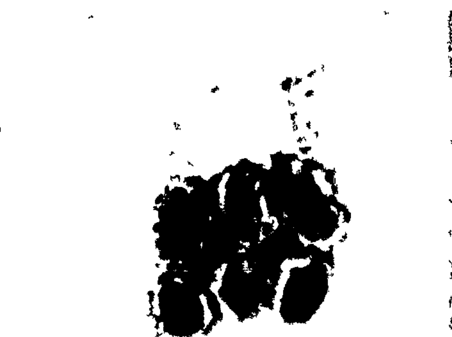
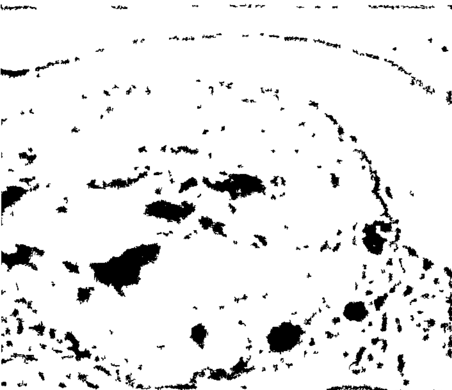
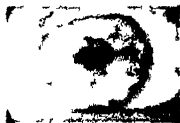
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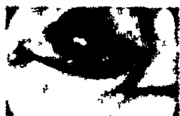
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THE TRANSPLANTATION OF TISSUES AND ORGANS







THE TRANSPLANTATION OF TISSUES AND ORGANS

MICHAEL F. A. WOODRUFF, M.D., M.S.(MELB.)
F.R.C.S., F.R.C.S.E., F.R.A.C.S.



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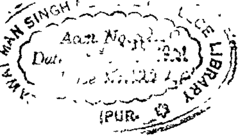
CHARLES C THOMAS · PUBLISHER
BANNERSTONE HOUSE
301-327 East Lawrence Avenue, Springfield, Illinois, U S A.

Published simultaneously in the British Commonwealth of Nations by
BLACKWELL SCIENTIFIC PUBLICATIONS, LTD , OXFORD, ENGLAND

Published simultaneously in Canada by
THE RYERSON PRESS, TORONTO

Library of Congress Catalog Card Number 59 15615

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Preface

Acknowledgments

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*Omission of the author's name indicates that he is no longer living.

Contents

THE TRANSPLANTATION OF TISSUES AND ORGANS

CHAPTER 1

Basic Principles and Techniques

...ferred to as skin grafts and bone grafts ...ely

...ount from which a transplant is ob... a *donor*, an animal to which ... transferred is called a *recipi*... When, as often happens, it is ... distinguish between tissue ...ives from a transplant and tis... not, the term *host tissue* is ... only the latter, even in the special case where the same animal is used both as donor and as host.

In speaking of animals we include, of course, human beings. As stated in the introduction, however, we shall have little to say about experiments in lower forms and throughout the book, except where the contrary is stated, the words transplantation and grafting refer to transplantation and grafting in mammals.

CLASSIFICATION OF TRANSPLANTS

...lants may conveniently be classi... the following ways

1 ...utologous, isologous, homologous ...logous transplants

...utologous transplant or *autotrans*... transplant made to a new site in ... ...dual

... transplant or *isotransplant* ... from one individual to ... or almost isogenic) with ... human beings this implies ... host are identical twins ... the donor and host ... twins or members ... strain (see however

...ansplant or *homotrans*... made from one human ... from one animal to ... species, excluding the ... and host are isogenic.

...transplant or *heterotrans*... ...ant from an animal to man ...mals of different species

... noted that autotransplanta... being performed deliberately, ... spontaneously. Familiar exam... ...spontaneous autotransplantation are the descent of the testis during intra-uterine life, the phenomenon of endometriosis, and the metastasis of malignant tumours. The developing foetus may be regarded as a special kind of homotransplant (*see* Chapter 10).

2 *According to the organ or tissue transplanted.*

Under this heading it is necessary to distinguish between normal and neoplastic tissue, and in the former case between tissue from an adult, an immature animal and a foetus. In the case of transplants of pieces of tissue, as distinct from whole organs, the quantity of material transplanted should be specified.

3 *According to whether the material is transplanted directly from the donor or after a period of storage in vitro.*

Stored transplants may be further subdivided according to whether the tissue is maintained in a condition of active life in tissue culture, kept alive but relatively inactive at low temperature, or killed.

4 *As free transplants, pedicle transplants or transplants by vascular anastomosis.*

A *free transplant* consists of an isolated piece of tissue which, at the time of transplantation, is completely devoid of vascular and nervous connexions. Sometimes multiple free transplants are used, and on occasion these take the form of isolated cells, or small clumps of cells, in suspension.

A *pedicle transplant* remains connected to the donor site, at least for a time, by a pedicle containing blood vessels. When a

OPERATIVE TECHNIQUE*

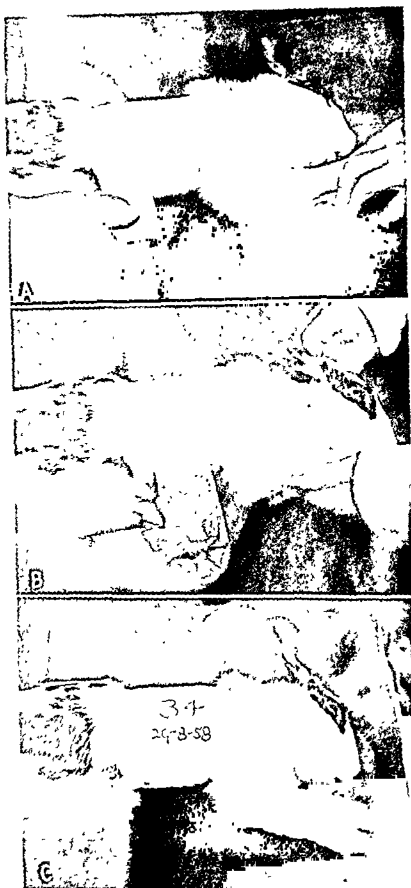


Fig 1. Dressing technique for small animals using open wove bandage (A) surmounted by one or two turns of plaster of paris bandage (B, C).



Fig. 1 Dressing technique for small animals using open wove bandage (A) surmounted by one or two turns of plaster of paris bandage (B, C)



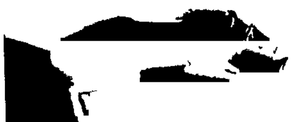


Fig 2 Dressing technique for small animals using self adherent crepe rubber bandage

animals with dressings should be kept in individual cages

FREE TRANSPLANTS

Orthotopic Skin Grafts

Orthotopic skin grafts may take the following forms.

1 Full thickness grafts.

2 Split skin grafts, consisting of the epidermis and part of the dermis

3 Pinch grafts, that is, small grafts which are full thickness in the centre but thinner at the periphery

4 Epidermal sheets

5 Epidermal cell suspensions

Full thickness grafts and pinch grafts are usually cut freehand with a knife or with scissors (*see* Billingham and Medawar, 1951) Split skin grafts may also be cut freehand but for cutting large split grafts it is often convenient to use a special appliance known as a dermatome (Chapter 12) One type of dermatome intended for use in children but which has proved very satisfactory in rats (Woodruff and Simpson, 1955a) and other animals, is illustrated in Figure 3, but various other types are available

Epithelial sheets may be prepared by incubating split skin grafts for an hour in a buffered solution of commercial trypsin at 37°C (Billingham and Medawar, 1951; Billingham and Reynolds, 1952), this digests the elastic material of the basement membrane, which plays an important part in anchoring the epidermis to the dermis, and allows the epidermis to be stripped off in a continuous sheet

To prepare epidermal cell suspensions the period of incubation with trypsin is slightly prolonged The epidermal sheet is then rinsed in physiological saline, and a solution of 0 8 per cent sodium citrate in saline is applied to the deep surface After a few minutes epidermal cells from the Malpighian layer can be scraped off with a knife and suspended in citrate solution

Skin grafts other than epidermal cell suspensions are best transplanted to a fresh raw surface on the thorax or back prepared by removing an area of skin down to the level of the vascular plane overlying the panniculus carnosus muscle It is easy to prepare such beds in rabbits, but rather more difficult in guinea pigs, rats and mice, because in these animals there is no well defined plane of cleavage at the required level A useful technique is illustrated in Figure 4

Sometimes a fitted graft (Medawar, 1944) is used, that is a graft which exactly fits the defect, alternatively open style grafts are used (Fig 5) Fitted grafts may or may not be sutured in place

Epidermal cell suspensions may be grafted to raw areas prepared as described above, in guinea pigs, however, where pigmented cells (melanocytes) may be grafted to a non pigmented area and hence readily identified, it is more satisfactory to use a 'half thickness graft bed" (Billingham and Medawar, 1951, Billingham and Sparrow, 1954).

After free skin grafting dressings are used



[illegible] a split skin graft from a rat with a Padgett-type dermatome. C. Removing the graft [illegible] D. Hist[illegible] section of graft [illegible] The scale denotes thousandths of an inch. [illegible] *Reconstructive Surgery* by courtesy of the publishers.)

[illegible] to ma[illegible] firm contact betw[illegible] and bed [illegible] limit loss of [illegible] from [illegible] to protect the grafts [illegible] contamin[illegible] and injury. A con[illegible] met[illegible] dust the area lightly [illegible] penicill[illegible] powder, cover with [illegible] impregnated with petroleum jelly [illegible], and then use an ordinary bandage *followed by a plaster bandage* (Medawar 1944 Billingham and Medawar, 1951), or one thickness of crepe rubber bandage, as already described. Various modifications have been suggested. The author has sometimes found it convenient to use a layer of cellophane, well smeared with sterile petroleum jelly, between the graft and the tulle gras. Taylor and Lehrfeld (1953) apply Terramycin ointment to the graft, cover with sterile gauze and bandage, and then use an *outer protective layer made of thin* sheet aluminium. Medawar and Woodruff (1958) developed a technique for use in

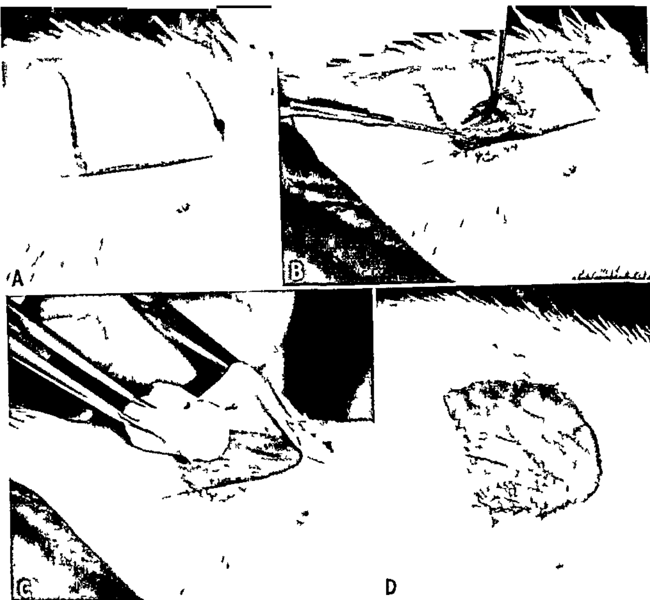


Fig. 4. Preparing a bed for a skin graft. A. Area outlined by incisions. B, C. Dissecting off the skin. D. Note the vessels on the panniculus carnosus which have been carefully preserved. (Reproduced from *Plastic and Reconstructive Surgery* by courtesy of the publishers.)

new born rats (Fig. 6) in which a single small split skin graft was kept in place by a circular piece of adhesive strapping separated from the graft by a smaller disc of sterile filter paper.

Baker (1941) has devised a technique which avoids the use of conventional dressings and uses the recipient's skin for this purpose. He reflects a flap of skin, places the graft on the defect thus created, and then sutures the flap back over the graft. Later, when the graft is well established, he excises the overlying skin.

Orthotopic Transplants of Other Tissues

The techniques used for orthotopic transplantation of connective tissue, nerve, bone, and cornea in experimental animals are similar to those used in clinical surgery; they are described in Chapters 15, 17, 18 and 20 respectively.

The transplantation of blood vessel seg

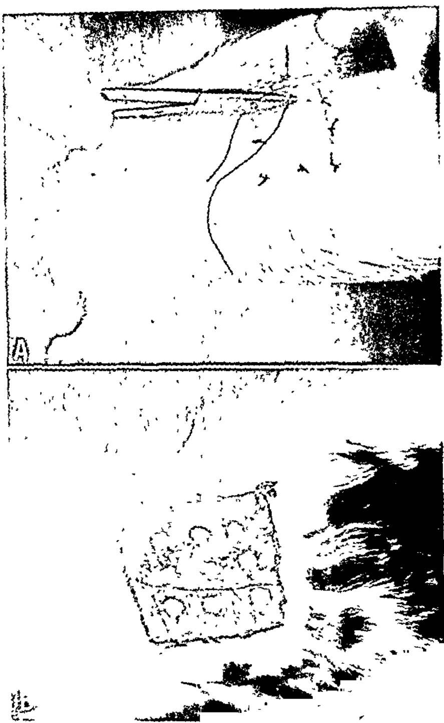
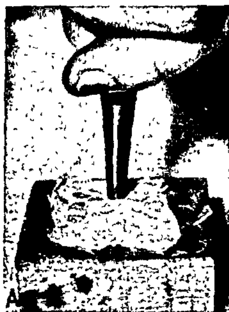


Fig 5 A Fitted graft B Open style grafts

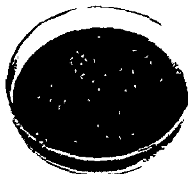
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Fig. 6 Skin grafting newborn rats A, B Preparing the grafts C Dressings consisting of a circular piece of adhesive strapping carrying a small disc of sterile filter paper. D, E. Preparing the bed The animal is anaesthetized with ether and immobilized by a piece of adhesive cellulose tape Note the vessels on the panniculus carnosus F, G. The operation is completed by placing the graft on the bed and applying the dressing

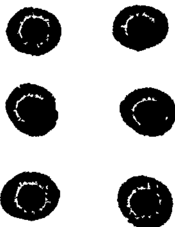

A

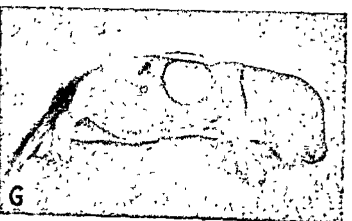

D


E


B


F


C


G

ments will be considered briefly with the transplantation of organs by vascular anastomosis (v. infra). Further details will be given in Chapter 21.

Heterotopic Transplants

General Methods for Transplanting Small Pieces of Tissue

Transplants of small pieces of tissue ... ed into various sites through a ... wound by means of a needle or a Pasteur pipette. The procedure is simple and several techniques are available (Fig. 7) to suit different circumstances.

When, however, a transplant has to be placed accurately in relation to some important structure, it is better to expose the site by open operation and to insert the tissue with forceps under direct vision.

Transplantation to two sites, the anterior chamber of the eye and the brain, requires special mention.

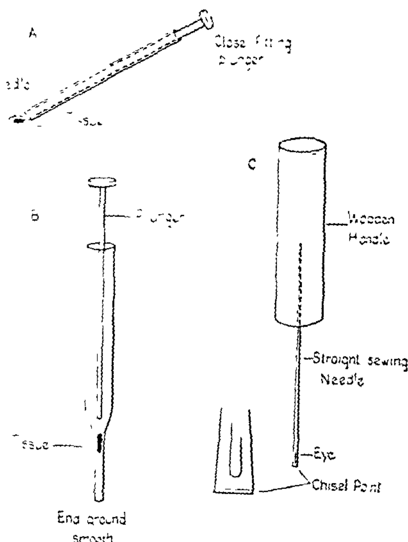


Fig. 7 Instruments for inserting small transplants. A Hollow needle with close fitting plunger showing method of loading. B. Pasteur pipette and plunger C Cherry's needle, made from an ordinary straight sewing needle by inserting the sharp end in a wooden handle and grinding a chisel edge on the other end The transplant is placed in the eye of the needle.

Transplantation to the Anterior Chamber of the Eye

Either local or general anaesthesia may be used. Satisfactory local anaesthesia may be obtained by instilling drops of 2 per cent cocaine ten minutes before the operation. Greene (1938, 1947) has described three basic steps in the operation: first, steady the eyeball and make a small incision at the corneo-scleral junction, secondly, insert the tissue through the incision, thirdly, by gentle pressure on the cornea displace the transplant to a point in the angle between iris and cornea diametrically opposite the incision.

Greene himself makes the incision with a double-edged knife and inserts the transplant either with forceps, or by means of a needle and plunger. He and his colleagues have used the technique widely in rabbits (Greene, 1938, 1942a, 1943), guinea pigs (Greene, 1941a, 1942a, 1943, 1946) and mice (Greene, 1946, 1947). Woodruff and Woodruff (1950), working with guinea pigs, found it best to steady the eyeball by grasping a fold of conjunctiva with forceps, make the incision with a special glass instrument (Fig. 8) and insert the tissue by means of a Pasteur pipette and plunger (Fig. 9). Polunin (1950) manipulates the transplant on the end of a Pasteur pipette, holding it in place by suction. Yet another method is to impale the transplant on the end of a dental pulp reamer.

The small corneal incision heals rapidly and no special after-treatment is required. The main difficulty in the operation is to avoid injuring the iris or allowing the transplant to slip behind the iris into the posterior chamber.

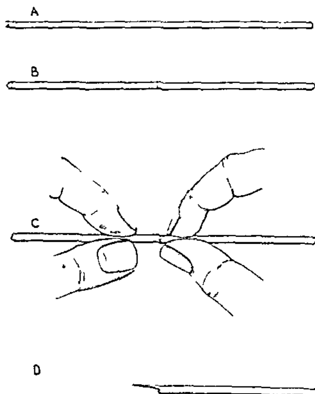


Fig. 8. Method of making glass knives for use in transplantation to the anterior chamber. A. Glass tubing drawn to about 2 mm. external diameter. B. A transverse scratch around half of the circumference has been made with a diamond. C. Breaking the tubing by shearing. Note the position of fingers and thumbs in relation to the scratch. D. The finished knife.

Transplantation to the Brain

The simplest method is to make a small incision down to the skull, drill a hole in the bone with a dental bur, and insert the tissue into the brain with a Pasteur pipette and plunger.

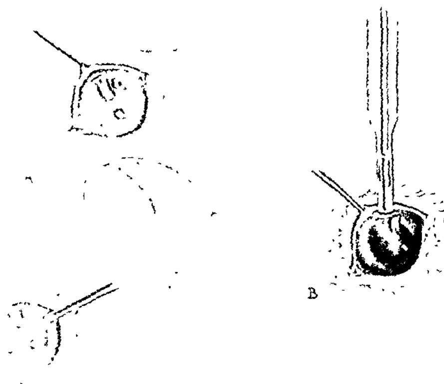
Preparation and Injection of Cell Suspensions

Cell suspensions are most commonly prepared from spleen, bone marrow and tumours, but other tissues are sometimes used.

The composition of the fluid in which the cells are suspended is important. Ringer's solution buffered to pH 7.4 with bicarbonate or 'tris' is often adequate but if the highest possible yield of viable cells is required it may pay to use one of the more complex saline solutions such as Tyrode's solution,* Hank's solution* or Gey's solution*, with or without the addition of 10 per cent autologous, homologous or heterologous serum, or alternatively pure serum.

If the material is to be injected intra-

*See Parker (1950) and Hanks (1948) for the composition and preparation of these solutions.



[illegible] plantation to the anterior chamber of the eye A Globe steadied by forceps [illegible] incision in upper part of cornea close to limbus. B Graft inserted by [illegible] and plunger C Graft displaced to a position diametrically opposite the [illegible] by stroking the cornea gently with a smooth rod.

[illegible] get rid of large [illegible] risk of pulmo- [illegible] other hand the [illegible] too roughly or [illegible] found to be non- [illegible] to grind the [illegible] with the suspend- [illegible] homogenizer (Fig. 10) [illegible] through stainless steel mesh, but it seems equally satisfactory to rub the tissue through the mesh without using a homogenizer. High speed electric blenders should not be used as they cause far too much damage to the cells Even when great care is taken intravenous injection of cell suspensions to new born animals may cause sudden death, but this may be avoided by washing the cells in Ringer-phosphate solution containing heparin 10 i.u./ml., thus eliminating cytoplasmic particles and cellular debris (Billingham and Brent, 1957a).

When the suspension has been prepared it should be sucked up into a syringe through a needle of the size to be used for the injection The number of cells per c mm. may be determined by diluting a sample of the suspension with 2 per cent acetic acid and counting with a haemocytometer

A suspension of lymphocytes can be obtained very easily in rats by cannulating the thoracic duct as described by Gowans (1957).

Subcutaneous, intramuscular and intraperitoneal injections are easy to make and call for no special comment.

Intravenous injections are normally given into the marginal ear vein in rabbits. The same procedure can be used in guinea pigs though it is much more difficult and it may pay to expose the jugular vein by open operation. In adult rats and mice one of the veins of the tail is generally used but some workers find it easier in rats to expose the jugular vein, especially if a measured quantity of suspension is to be injected. In newborn rats and mice intravenous injections can be made with a 30 gauge needle into the orbital branch of the anterior facial vein as described by Billingham and Brent (1957a), a modified form of whose technique is illustrated in Figure 11.

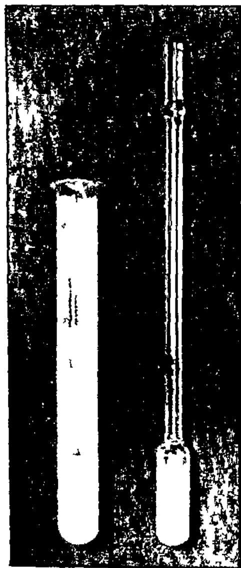


Fig 10. Glass homogenizer for preparing cell suspensions

ANASTOMOSIS AND TRANSPLANTATION OF BLOOD VESSELS

Blood vessels may be anastomosed by suture or by a variety of non-suture techniques.

Anastomosis by Suture

Effective techniques for anastomosing blood vessels by suture were developed during the early years of the present century principally by Alexis Carrel and C. C. Guthrie.*

The actual operative procedures have since undergone only minor modifications, but the introduction of anticoagulants (p. 19) has greatly increased the prospect of success in anastomosing small vessels.

The anastomosis may be made end to end, end to side, side to side, or by the patch method. The suture material is usually fine (5/0) silk impregnated with liquid paraffin. It is best to use *atraumatic sutures* consisting of a length (about 30 in.) of silk swaged to an eyeless needle, which can be purchased ready sterilized in ampoules, but these are expensive and ordinary threaded needles are sometimes used in experimental work.

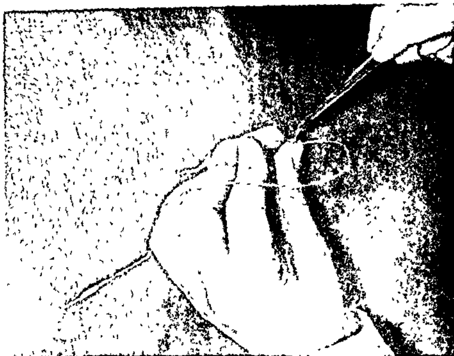
End to End Anastomosis

We shall consider first, as an example, simple division of an artery and restoration of continuity by end to end anastomosis.

Special clamps are applied to the host artery, one proximal and the other distal to the point of section. Bulldog clamps are often used for this purpose but more elaborate clamps such as Crile's arterial clamp, Blalock's arterial clamp, and Potts' ductus clamp are preferred by some operators (Fig. 12). The author finds Potts' ductus clamp very satisfactory; its fine teeth prevent slipping and do not seriously damage a healthy artery if the clamp is not tightened unduly.

The artery is divided cleanly with a sharp knife and the open ends are washed out

*The history of blood vessel anastomosis and transplantation is discussed in detail in Chapter 21.



11 Intravenous injection of newborn rats using the author's modification he technique of *Billingham* and *Brent* The syringe and needle are con-ted by a length of fine polyethylene tubing The operator inserts the dle and then holds it steady while the syringe is operated either by an assistant or by a machine controlled by foot switch

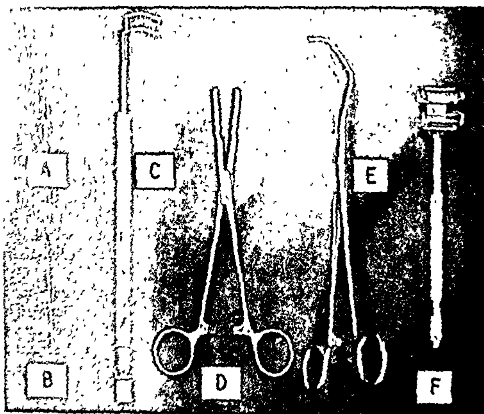


Fig 12 Clamps for blood vessel anastomosis. A Bulldog clamp B Crile clamp. C Blalock clamp D Potts ductus clamp E, F Clamps used to occlude portion of the lumen of a large vessel.

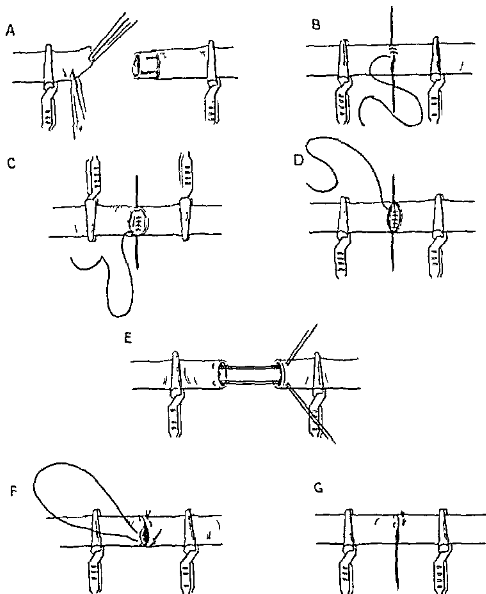
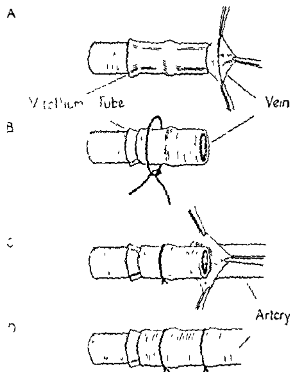


Fig 13 End-to-end blood vessel anastomosis A Excision of adventitia B C D Over-and-over stitch using two traction sutures After the first half of the continuous suture (B) has been inserted the vessel is rotated through 180° to enable the anastomosis to be completed (C) When the vessel cannot be rotated the first half of the suture is inserted from within the lumen (D) but the knots are tied on the outside E F Continuous everting stitch using two traction sutures G Anastomosis by interrupted sutures

with saline to which a little heparin (10 i u per ml) has been added A cuff of adventitia is excised on each side (Fig 13A) if this is not done tags of adventitia may be accidentally included between the ends of the vessel when they are sutured

Various methods of suture are illustrated in Figure 13

When the vessel can be rotated two (as illustrated) or three traction sutures are inserted at equidistant points of the circumference and tied By traction on these sutures the circular outline of each vessel is converted into a slit or an equilateral triangle The contiguous edges are joined in turn by a continuous over-and-over stitch (Fig 13B C) a continuous everting stitch (Fig 13F) or interrupted stitches (Fig 13G) the vessel being rotated so that the edge being sutured lies uppermost



[illegible] Anastomosis of an artery to a vein graft [illegible] Wakefield Lord prosthesis. One vessel [illegible] through the prosthesis (A) and everted [illegible] prosthesis thus prepared is then inserted [illegible] other vessel (C) and secured by [illegible] ligature (D)

[illegible] this alone does not always suffice [illegible] vessels anastomosed are of small [illegible] such cases the chance of success [illegible] much increased by anticoagulant [illegible]

[illegible] coagulant drugs are of two main k[illegible] *heparin*, which acts quickly by inactivating thrombin in the blood stream, and drugs like *dicoumarol, tromexan* and *dindevan*, which act slowly by inhibiting the formation of prothrombin in the liver. Administration of heparin is controlled by determination of *the clotting time* of the blood, administration of *the other drugs* by determination of the *prothrombin time*.†

*It is particularly important to avoid crushing the vessel with forceps or clamps, to wash out any clot in the ends of the vessel and to use paraffin impregnated sutures.

†For details regarding these tests *see* Biggs and Macfarlane (1957)

It is possible to use heparin alone, but the drug is very expensive and has to be given by injection. It is cheaper, and just as effective, to use a combination of drugs. A suitable regime in dogs is as follows:

Before operation: Determine the prothrombin time. Give dicoumarol 1.5 mg. per kg. body weight by mouth a few hours before operation.

During operation: Inject 5000 units of heparin into the main vessel proximal to the anastomosis just before finally removing the clamps.

After operation: On the morning after operation, and each morning thereafter for a period ranging from a few days to a fortnight, determine the prothrombin time and give a maintenance dose of dicoumarol sufficient to keep the prothrombin time at about twice the normal value. On average about 1 mg. per kg. is required.

The disadvantage of using anticoagulants, especially dicoumarol and allied drugs, is that *haemorrhage occasionally results*, usually from small vessels divided in exposing the artery to be operated on rather than from this vessel at the site of the anastomosis. When heparin alone is used the abnormal tendency to bleed may be abolished by injecting *protamine*, but when the other drugs are used the only effective measure is blood transfusion. In any given experiment, therefore, it is wise to make a few trials to determine whether sufficient gain results from anticoagulant therapy to justify the risk of haemorrhage.

Blood Vessel Grafts

Blood vessel grafts are of two main kinds: grafts in continuity and bypass grafts (Fig. 18). To insert a graft in continuity a segment of the host vessel is isolated between clamps, excised, and replaced by the graft. Two anastomoses are required and both are usually end-to-end. When a bypass graft

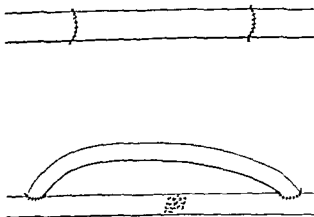


Fig 18 Diagram illustrating an arterial graft in continuity and a bypass graft

is used, on the other hand, both anastomoses are end to side

In grafting to the aorta, especially in its thoracic part*, it may be dangerous to arrest the blood flow completely for the length of time required to suture the graft In dogs, for example, complete occlusion of the previously unobstructed† thoracic aorta for more than ten or fifteen minutes is prone to result in spastic paralysis (Carrel, 1910b Blalock and Park, 1944)

Sufficient time for the grafting operation may be obtained if the circulation is temporarily maintained by a shunt, or if the animal's temperature is reduced by artificial hypothermia. These methods are fully described in Chapter 21.

*In sheep Borrie and Woodruff (1955) have found that occlusion of the abdominal aorta just below the renal arteries for 30 minutes results in permanent paraplegia In human beings occlusion of the common carotid artery may cause hemiplegia

†Where there has been preceding partial obstruction the effect of complete obstruction is less marked Thus complete occlusion of the human aorta during Crafoord's operation (Chapter 21) for coarctation is well tolerated

TRANSPLANTATION OF ORGANS BY VASCULAR ANASTOMOSIS

With the development of satisfactory methods of blood vessel anastomosis it became possible to transplant *not only blood* vessel segments but also whole organs including the thyroid, adrenal, ovary, kidney and spleen, and this list has now been extended to include the lung and the heart.

In principle what is required is to anastomose the main artery and vein of the transplant end to end or end to side to suitable host vessels, and in the case of organs such as the kidney having an external secretion to provide for its disposal In practice many technical problems have to be overcome, and these are discussed in detail in Chapters 23 26 inclusive

PEDICLE AND PARABIOTIC TRANSPLANTS

Autologous pedicle skin grafts are used in surgery, and also, but to a lesser extent, in experimental work The technique is essentially the same in both cases, and is described in Chapter 14

Parabiosis, and parabiotic skin and organ transplants, are considered in Chapter 3.

THE FATE OF TRANSPLANTS

Transplants of Living Tissue

The life of a transplant is, of course, terminated by death of the recipient animal unless the transplant is transferred to another host Accurate comparison of the properties of different types of transplant is therefore possible only if the recipients survive long enough for differences in behaviour to become apparent. Assuming this to be the case we may distinguish four possibilities

1 *The transplant becomes established and survives permanently.*

Claims of permanent survival should be made only if the following criteria are fulfilled.

(1) The transplant survives throughout the life of the recipient.

recipient survives for a long time
at least a year
examination of the trans-
of the recipient gives no
supposing that the transplant
destroyed had the recipient

do not necessarily imply that
of the transplant survives
may properly be applied
transplant survives, or cells
type survive or if, as some-
much of the transplant is
the lost tissue is subsequently
generation from the surviving

indefinite survival is some-
synonym for permanent

transplant is systematically re-
living host tissue
none of the original
the gross appearance
erroneously that the
This "creeping
characteristically with
of bone (Chapter 18).
for a time but
leaving either no
tissue, which may or
remnants of the

that the transplant be-
but undergoes secondary

transplant is rapidly destroyed
event we may say that the trans-plant fails to become established or that it undergoes primary destruction.

The distinction between primary and secondary destruction, though convenient, is somewhat arbitrary. We may, for example, agree to say that a transplant has become established if, and only if, it has become vascularized, or, alternatively, if it survives for longer than some stated period; but in practice neither of these criteria is satisfactory. The first is misleading because a transplant of an avascular tissue such as cartilage or cornea may survive for a long time without ever becoming vascularized, whereas an organ transplanted by vascular anastomosis—and hence established according to this criterion—may not survive for more than a few days; the second is unsatisfactory because the behaviour of different tissues varies so greatly that no generally applicable time limit can be assigned.

Non-living Transplants

With transplants of dead tissue there are three possibilities:

1. *The dead material remains in situ for a long period.*

This sometimes happens, for example, with heterotransplants of merthiolate-preserved cartilage.

2. *The dead material is systematically replaced by living host tissue.*

This occurs, for example, with freeze-dried or deep frozen transplants of bone and of arteries.

3. *The dead material is rapidly absorbed or discharged.*

CRITERIA OF SURVIVAL

Evidence of the survival of a transplant of living tissue may be based on tests for functional activity or on biopsy findings.

Tests for Functional Activity

Tests for functional activity can be applied most readily to transplants of glandular tissues and organs. They are of two main kinds.

In the first place we may test directly the functional activity of the transplant. For example we may study the output of fluid

and electrolytes from a transplanted kidney, or the uptake of radioactive iodine by a transplant of thyroid Tests of this type are ideal in theory but may be difficult to apply in practice

Secondly we may apply tests to the host as a whole and from these deduce the degree of activity of the transplant Such tests are used mainly with endocrine transplants and are particularly useful when there has been a gross endocrine deficiency prior to transplantation Thus for example, after transplantation of adrenal tissue to an adrenalectomized recipient we may employ Thorn s test (Thorn, Forsham Prunty and Hills, 1948) and base our assessment of adrenal function on the effect of adrenocorticotrophic hormone on the level of circulating eosinophils and after transplantation of ovarian tissue to ovariectomized rats and other animals we may study vaginal smears to determine when, if at all, the oestrous cycle is restored Tests of this type are often convenient in practice, but are likely to be misleading if the assumption that the host has no tissue of its own of the kind in question turns out to be erroneous This point needs emphasizing because if in seeking to remove an endocrine organ completely, tiny pieces are accidentally left behind or areas of accessory tissue are overlooked hypertrophy may subsequently occur and result in a high level of functional activity

Tests Based on Biopsy

Biopsy of a transplant implies removal of the whole or part of the transplant together with some of the adjacent host tissue Removal of the whole transplant is usually the more reliable procedure because the structure may be far from uniform but has the disadvantage when there is only one transplant that it terminates the experiment If, however, several pieces of tissue, as nearly as possible identical are transplanted in one operation and serial biopsies are performed at intervals, the period of survival under the conditions of the experiment may be given a precise meaning and determined within reasonably narrow limits (Medawar, 1944, Darcy, 1952)

The simplest test is to examine the biopsy specimen histologically A single section is often misleading and it is usually best to cut up the whole block and examine sections spaced at equal intervals across it This is a widely used and valuable procedure, but it does not always suffice to distinguish between survival of a transplant and creeping replacement by host tissue

Secondly, an attempt may be made to grow the material obtained at biopsy in tissue culture This is not a very satisfactory procedure firstly because the morphological appearance of specialized cells often changes radically in culture and secondly because the cells which migrate from the explant are often predominantly fibroblasts and it is difficult to determine whether these arise from the transplant proper or from adherent host tissue On the other hand, in investigating possible methods of storing tissues prior to transplantation, explantation in tissue culture has proved to be a useful test for the presence of viable cells (Peirce, Gross, Bill and Merrill, 1949), especially with transplants of blood vessels and bone

Finally, the biopsy specimen may be transplanted back to the original donor This is the most certain test of survival It has been used by Medawar (1948a) in studying experimental transplants of skin, and by Mitchison (1955a) in timing the survival of tumour homotransplants

CHAPTER 2

Autotransplants

A[illegible]nsplantation may occur spon[illegible] may be performed deliberately [illegible] experimental or therapeutic proce[illegible]

[illegible] most familiar example of spontane[illegible] [illegible]nsplantation is the metastasis of [illegible]nant tumours, but the process may also [illegible] with non neoplastic tissue and is re[illegible] for such pathological conditions as endometriosis, pseudomyxoma peritonei, and splenosis following traumatic rupture of the spleen (McCann, 1956). There is, moreover, some evidence (p. 153) that cells concerned in haemopoiesis may under certain conditions enter the blood stream and be carried to new sites where they resume their normal function, and it is conceivable that this is a normal physiological process.

CONDITIONS NECESSARY FOR SURVIVAL

[illegible]nts which once become [illegible] commonly, though not invari[illegible] permanently The conditions [illegible] survival may be considered [illegible]dings' conditions necessary for [illegible]val of autotransplants in general, [illegible] special requirements of particular tissue[illegible] organs.

General Conditions

[illegible]althy Tissue

No transplant is likely to survive if it has [illegible]een severely damaged, for example by gross [illegible]echanical [illegible]jury or by storage under un[illegible]itable co[illegible]tions.

Freedom from Infection

A transplant is unlikely to survive in the presence of severe infection, and even a slight degree of infection may prove lethal.

Adequate Nutrition

If a transplant is to survive it must be provided with an adequate supply of nutrient material and adequate facilities for the removal of waste products. With transplants by vascular anastomosis a blood supply is assured *ab initio* and there is no immediate problem, though subsequently thrombosis may occur and lead to death of the transplant With free transplants metabolic exchange depends initially on the diffusion of fluid to and from the surrounding tissue. Transplants of avascular tissues such as cartilage and cornea may continue to be nourished in this way indefinitely, and the same is occasionally true of very small* transplants of tissues which are normally vascular Usually, however, survival of a free transplant of a tissue which is normally vascular is conditional on the transplant acquiring, sooner or later, a blood supply.

If, as sometimes happens, the peripheral

*Earle (1934b) sets the upper limit at 1 mm. diameter for transplants of tissues which have a "reasonably high" oxygen consumption.

part of a transplant survives but the central part undergoes avascular necrosis, the ultimate size and structure of the transplant depend on whether or not the tissue is capable of regeneration

A distinction is sometimes drawn between viable and non viable transplants, but the *meaning of these terms is not always clear* We shall define a viable transplant as one which is capable of surviving in a suitable environment, and a non viable transplant as one which is incapable of surviving in any environment Non viable transplants thus include, in addition to transplants which have been severely damaged, free transplants of healthy tissue which, on account *of their excessive size or metabolic activity*, will inevitably perish before there is time for an adequate blood supply to develop

Special Requirements of Particular Tissues
Hormonal Stimulation

Free transplants of endocrine tissues are sometimes more successful if the recipient animal is grossly deficient in the tissue in *question at the time* of transplantation This generalization is often referred to as Halsted's law of deficiency, though it was suggested first by Cristiani (1903b, 1905a, b) in respect of thyroid transplants in rats Cristiani's conclusion, though not necessarily untrue, was unjustified because in some experiments he compared autotransplants in thyroid deficient animals with homo transplants in intact animals Halsted (1909) based his conclusion on studies of auto transplants of parathyroid in dogs in which he found that survival occurred only if the recipient had been deprived of at least half of its parathyroid tissue

The validity of Halsted's law has subsequently been tested by many workers and their findings are discussed at length in Chapter 23 Critical examination of Halsted's own protocols shows that they are not really conclusive, and according to Shambaugh (1936) the law does not, in fact, hold good for parathyroid transplants It does appear to be true in the main for transplants of adrenal cortical tissue (Wyman and Tum Suden, 1932, 1937a, Ingle and Higgins, 1938a), and for transplants of thyroid (Ingle and Cragg, 1939), especially those made to the anterior chamber of the eye (Woodruff and Woodruff, 1950), but transplants sometimes survive when the deficiency is minimal (Everett, 1949)

It would seem that it is not the adrenal or thyroid deficiency *per se* which is important, but the increased production of pituitary corticotrophin and thyrotrophin respectively which these deficiencies evoke, since comparable transplants do survive in animals with minimal deficiency if the appropriate hormone is administered (*see* Wyman and Tum Suden, 1937a, Woodruff and Woodruff, 1950)

Free transplants of adrenal characteristically undergo massive central necrosis, and the same is true of free transplants of other endocrine tissues, successful transplantation is therefore contingent on regeneration from surviving peripheral cells With adrenal and thyroid tissue such regeneration does not occur in the absence of the appropriate hormonal stimulation

Hormonal stimulation may also play an important part in determining the survival and regeneration of transplants of the gonads, uterus, mammary gland and prostate

Nervous Stimulation

An intact nerve supply appears to be necessary for the survival of transplants of skeletal muscle (Chapter 16) Thus free transplants, though they may survive for several weeks and may even show regeneration (Shinya, 1911), eventually regress and are replaced by fibrous tissue (Volkmann, 1892), whereas muscle pedicle transplants, *in which nerves as well as vessels are retained* intact may survive permanently (Erlacher, 1915) This phenomenon is not peculiar to adult muscle It has been shown, for exam

ple that non-innervated muscle in free transplants of limb buds in chick embryos ... to differentiate, undergoes fatty degeneration and is soon absorbed; whereas if the transplant includes part of the neural ... that the muscle is innervated, differ... ...tinues and there is little or no ... Hunt, 1932; Eastlick, 1943). ... to which the behaviour of ... transplanted autologously by ... stomosis is modified by the in... ...ruption of their nerve supply ... in Chapters 25 and 26

Mechanical Stimulation

Stimu... by subjection to mechanical ... to be necessary to ensure regen... ... transplants (Chapter 18), ... this stimulus is probably the ... why bone transplants which ... surrounded by soft tissues tend ... characteristic structure and, if ... appear completely

... Temperature

... temperature is an important ... ...cular transplants It has ... that in cryptorchidism ... does not occur in the mis... ... and in 1922 Crew suggested ... because except in the scrotum, ...ciently air cooled, the local tem... is too high Moore (1922, 1924a, ... investigated the matter experimentally ... pigs, rabbits and other animals, and his findings, which are discussed in detail in Chapter 24, support Crew's hypothesis

It is conceivable that the local temperature is critical for transplants of other tissues and organs besides the testis. It has been found, for example, that autotransplants of thyroid in thyroidectomized guinea pigs, which are able to survive and regenerate in many sites, regress if transplanted subcutaneously to the external ear (Woodruff, unpublished experiments), whereas autotransplants of adrenal cortical tissue apparently grow satisfactorily in the rat ear (Kroc, 1942); and it may well be that the thyroid transplants fail because the local temperature is too low.

Other Factors

Free autotransplants of small pieces of kidney, unlike autotransplants of whole kidneys by vascular anastomosis (Chapter 25), do not survive for long (Loeb, 1945; Dempster, 1953a). With free autotransplants of liver the peripheral cells give rise to new bile duct epithelium and also to liver cells, but the latter soon degenerate and atrophy (Cameron and Oakley, 1934). Portions of pancreatic duct transplanted beneath the serosa of the duodenum or jejunum in pancreatectomized dogs maintained on insulin may show some regeneration of ductules with formation of acini and islets (Shaw and Latimer, 1926), but subsequently regress

One factor, which was suggested by Shaw and Latimer to account for the failure of pancreatic transplants but which may be of general importance, is the lack of adequate provision for the escape of products of secretion. Another, which seems likely to be especially important with transplants of renal tissue, is the disturbance of function which necessarily occurs when part of a complex integrated structure is isolated. In the intact kidney, for example, each nephron is a functional unit and the tubules are regularly called on to deal with large volumes of glomerular filtrate; in a small free transplant, however, these conditions no longer obtain. With hepatic transplants lack of portal blood, with its high content of amino-acids and other substances and its low oxygen tension, may be important. This suggestion gains some support from the fact, reported many years ago by Mann and Magath (1922), that regeneration of the liver after injury does not occur in the presence of an Eck fistula. This is not easily reconciled, however, with the very curious

observation of Oakley (1938) that when homotransplants of embryonic chick liver are *made to the* chorio allantoic membrane of the developing chick embryo* liver cells survive readily but bile duct epithelium soon disappears

There are *doubtless many other factors* which have not been elucidated, and it would be illuminating to make a systematic *study of the* behaviour of free autotransplants of various tissues in different environments Such a study, especially if it *included* neoplastic as well as normal tissues, might throw some light on the factors which determine the distribution of tumour metastases Following Stephen Paget (1889) Willis (1948) and others have sought to explain the distribution in terms of the suitability of particular environments (or soils to use Paget s analogy) for particular types of tumour but, as Coman and his colleagues have pointed out (Coman, Eisenberg and McCutcheon, 1949), one must consider also the anatomical factors which help to determine the distribution of malignant emboli Admittedly only a proportion of these survive and proliferate to form true metastases, but there is no general agreement as to whether the ratio of surviving to non surviving emboli differs in different sites By studying experimental transplants instead of spontaneous metastases one variable is eliminated and it becomes possible to investigate the soil hypothesis directly

*This is a specially favourable site for heterotransplants (*see* Chapter 8)

THE STRUCTURE OF SURVIVING TRANSPLANTS

We may distinguish three cases

1 The bulk of the transplant survives intact though there may be some temporary *structural changes*

2 Many of the cells of the transplant are destroyed but the original structure is largely restored by regeneration from surviving cells

3 The structure is permanently altered because the transplant consists of cells of various kinds, some of which survive and proliferate while others do not

Survival of the bulk of the transplant following *temporary structural* changes occurs commonly with orthotopic autografts of skin This is illustrated by the behaviour of pinch grafts in rabbits, which has been described in detail by Medawar (1944) For a few days while the graft is becoming re*vascularized* there is mild traumatic inflammation, characterized by dilatation and engorgement of capillaries, migration of round cells from the vessels, and some oedema New collagen, possibly of host origin, is laid down in the dermis Epithelium spreads outwards from the graft and that forming the roof of the graft thickens, at first owing to swelling of cells and outward migration of epithelium from hair follicles, later due to intense proliferation of epidermal cells The hairs of the graft are shed but new follicles are formed After about eight days retrograde differentiation begins and the structure of the graft gradually returns to normal (Fig 19)

In man skin grafting is often undertaken under conditions less favourable than those used experimentally and the behaviour of the grafts is modified accordingly as described in Chapter 13 When, however, grafts are made to fresh raw surfaces having *an abundant blood* supply, the changes which occur are similar to those described above except that with thick grafts there may be conspicuous degenerative changes in the epidermis in the short period before the graft becomes vascularized

Restor*ation of the original structure* by regeneration from surviving remnants is characteristic of free transplants of thyroid



... skin in a rabbit 4 weeks after ... toxylin and eosin. × 100.

... conditions (*see* Ingle and ... eb, 1945, Woodruff and ... This is illustrated in Fig- ...

... alteration of structure due to ... certain types of cell to survive ... e transplants of adrenal gland ... With free adrenal transplants, as many workers have shown (*see* Jaffe and Plavska, 1926, Ingle and Higgins, 1938a, Woodruff and B...vell, 1953), the medulla and most of the cortex normally become necrotic * but under suitable conditions cortical tissue regenerates from a narrow zone of surviving cells at the periphery (Fig 21), the medulla, being incapable of regeneration, is lost permanently. This is in contrast to the behaviour of pedicle transplants of whole adrenals in which, as Dunphy and Keeley (1940) have shown in dogs, both the cortex and the medulla may survive. With free transplants of liver (to sites other than the chorio-allantoic membrane), as we have already noted, surviving peripheral cells give rise to bile duct epithelium and also to liver cells, but only the former survives for long

Cells which survive transplantation are, as a general rule, strictly true-breeding in their mitotic lineage and so retain their distinctive properties whether the transplantation is orthotopic or heterotopic. It has been shown, for example, that autotransplants of human cartilage, bone, tendon and skin retain their characteristic structure in different sites (Peer and Walker, 1951), and the same is true of these and many other tissues,

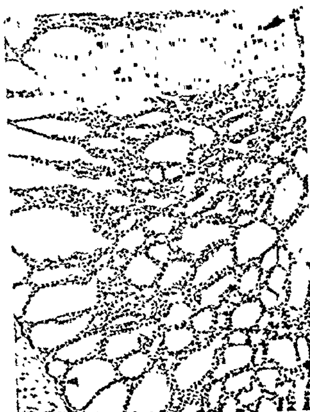


Fig 20 Free subcutaneous autograft of thyroid in a guinea pig 4 weeks after grafting, showing complete regeneration. Haematoxylin and eosin. ×100.

*With extremely small transplants necrosis may not occur, and Everett (1949) has reported survival, though not growth, of adrenal medulla in autotransplants about 0 3 mm diameter.



Fig. 21 Free autograft of a whole adrenal gland to the ovary of a rat 5 weeks after grafting. The cortex has regenerated and shows three distinct zones. The whole of the medulla has undergone necrosis and is in process of being replaced by connective tissue. Haematoxylin and eosin. ×100

including cornea (Billingham and Medawar, 1950a), *thyroid, parathyroid,* adrenal cortex and ovary in animals. Moreover with transplants of skin and related tissues the distinctive characteristics of the various races of epidermal epithelia are maintained (Billingham and Medawar, 1948a, b). In *guinea pigs for example, sole of foot skin,* body skin, claw forming epithelium and tongue epithelium retain their characteristic structure in any site to which they can be successfully transplanted. In man full *thickness grafts of hair bearing skin continue to grow hairs after being transplanted* to hairless regions and split skin grafts used in the vagina or the mouth retain the structure of skin.

An exception occurs when the cells of a transplant are subject to what Billingham and Medawar (1948b) have termed *infective transformation,* and this process, which is illustrated by the phenomenon of pigment spread in the skin of guinea pigs, will be discussed later (p. 33).

THE INTERACTION BETWEEN TRANSPLANT AND ENVIRONMENT

Transplantation by definition, implies a change of environment. The new environment however, is not static but changes continually so that we must think not merely of changes in the transplant but of the interaction between transplant and environment. This interaction is manifested in the vascularization of free transplants, in the phenomenon of tissue *induction,* and in the process of creeping substitution whereby a transplant is systematically replaced by regenerating host tissue.

The Vascularization of Free Transplants

The process of vascularization has been investigated mainly with skin grafts. The methods used, however, are for the most part applicable to other tissues and it will therefore be appropriate to consider them here. *They may be classified as follows*

1. Simple histological examination of grafts of various ages
2. Injection methods
3. Direct observation of the process of vascularization by means of the transparent chamber technique, or, in the special case of orthotopic skin grafts, by skin microscopy
4. The fluorescein test and other procedures designed for studying the revascularization of skin flaps (Chapter 14), but capable of being modified for use with free grafts

Simple Histological Examination

Simple histological examination may throw some light on the mechanism by which the nutrition of a transplant is maintained prior to vascularization; on the time [illegible] vascularization begins and, indi[illegible] the state of nutrition of a trans[illegible] any given moment. It is not, how[illegible] adequate method for studying the [illegible] of vascularization in detail.

[illegible]ation on Skin Grafts. A few min[illegible] grafting small vessels in the bed [illegible] and exudation of plasma begins. Leu[illegible] and histiocytes soon accumulate in [illegible] and some of the cell-containing [illegible] thus formed makes its way into the [illegible] vessels of the graft. This phe[illegible], which was first observed by Gold[illegible] and named by him "Die plas[illegible] Circulation," appears to play an [illegible] part in the early nutrition of the [illegible]

[illegible] studies of the vascularization [illegible] led to much difference of [illegible] the nature of the process. [illegible] who made the first important [illegible] this field, held that revas[illegible] depends largely, if not entirely, [illegible] of the graft by new vessels from [illegible] of which grow within the [illegible] the old vessels of the graft. The [illegible] of new vessels growing in from the [illegible] emphasized even more strongly by Goldmann (1894), who held that *all* the vessels of the graft degenerate. Braun (1899), on the other hand, believed that revascularization depends, at least in part, on anastomoses between vessels growing from the bed and the old vessels of the graft. Enderlen (1897) held intermediate views and maintained that the blood vessels of the graft degenerate but islets of endothelium survive and help to form new vessels; according to this view vascularization depends on ingrowth of vessels from the bed and on anastomoses between these and the new-formed vessels of the graft.

This controversy was not settled until other methods of investigation were developed.

Injection Methods

Important observations have been made by histological examination following injection of Indian ink and other simple colloidal suspensions to the living animal. It seems likely, however, that corrosion techniques and microradiology, which have proved so successful in studies of the renal circulation (Trueta, Barclay, Daniel, Franklin and Pritchard, 1947), would yield more detailed information. For corrosion preparations, neoprene latex, introduced by Lieb (1940), would seem to be the most suitable medium. The technique of microradiology has been fully described by Barclay (1947, 1951). Possible media include diodone, thorotrast, and suspensions of colloidal metals; of these the colloidal metals have the advantage that they may be used for histological as well as radiological studies.

Observations on Skin Grafts. Davis and Traut (1925) used an injection method to study the revascularization of buried full-thickness skin autografts in dogs. Grafting was performed at frequent intervals so that at the end of the experiment each animal had a series of grafts ranging in age from 1 to 40 days. Immediately after killing the animal Indian ink was injected into the heart. Half of each graft with the underlying tissue was cleared by the method of Spalteholz (1914) and serial sections were cut from the other half.

It was shown that revascularization occurs in two stages

The first stage begins as early as 22 hours after grafting and continues until about the end of the third day. During this time capillaries from the bed grow upwards and anastomose with pre existing capillaries in the graft. As evidence for this Davis and Traut point to the rapidity with which the graft becomes vascularized and the fact that

a vessel in the bed is often seen to be continuous with a slightly larger vessel in the graft.

During the second stage, which begins about the fourth day, the graft is invaded by capillaries growing into it from the bed Some of these new capillaries grow rapidly along the lumen of the pre existing vessels of the graft, others make their way more slowly through the connective tissue stroma. A circulation adequate to ensure survival of the graft is not established until the eighth day

Those small vessels of the graft which have anastomosed with capillaries from the bed during the first stage of vascularization survive, the rest of the small vessels and all the larger vessels of the graft disappear. The permanent circulation is derived largely from the vessels which invade the graft during the second stage of vascularization.

These findings have been confirmed by Mir y Mir (1951).

The Transparent Chamber Technique

The first successful technique for the microscopic study of cells and tissues *in vivo* over long periods of time was developed by Sandison (1924, 1928a, b), who devised a transparent chamber for insertion into the pinna of the rabbit Algire and his co workers (Algire, 1943; Algire and Legallais, 1949) modified the procedure for use in the study of transplantable tumours in the mouse, and more recently Conway and his colleagues (Conway, Joslin and Stark, 1951, Conway, Stark and Joslin, 1951a; Joslin, 1952) have further modified Algire's technique and used it to study the vascularization of skin grafts in mice In its final form, as described by Conway, Griffith, Shannon and Findley (1957), the apparatus consists of a framework of copper wire forming a "traction splint," and a chamber of cellulose acetate made in two halves and provided with a thin window of Saran plastic (a polymer of vinylidene chloride and vinyl chloride). The splint is applied to the mouse as shown in Figure 22, the separate pieces being held together with tantalum wire. For microscopic examination of the graft within the chamber the mouse is immobilized in a metal holder.

Conway's findings with skin autografts on the mouse may be summarized as follows:

Immediately after operation the chamber begins to fill with fluid The flow continues for about 24 hours and during this time the fluid becomes more cellular and more viscous Vasomotor control is disturbed by the operative trauma, as a result arterioles and meta arterioles are dilated in some parts of their length and contracted in others, and there is oscillation of blood in many vessels Multiple arteriovenous shunts may be seen The blood flow becomes stagnant and there is incipient thrombosis. After 4 6 days the capillaries in the tissue subjacent to and immediately surrounding the graft start budding, this process reaches its maximum 7-10 days after transplantation. Alternate filling and emptying of the capillary bed due to functioning of the "pre capillary sphincter" can be seen For some undetermined reason all vascular growth is directed towards the graft and after the twelfth day an areola of vessels around the graft can be seen macroscopically as well as microscopically.

Skin Microscopy

Skin microscopy provides a useful method for studying the vascularization of orthotopic skin grafts A drop of oil or liquid paraffin is placed on the graft and the examination is made with a binocular dissecting microscope using almost vertical illumination With small animals it is convenient to bring the graft into contact with the undersurface of the glass stage of the microscope; with large animals and human patients a thin glass plate is placed over the graft after the oil has been applied, and the micro-

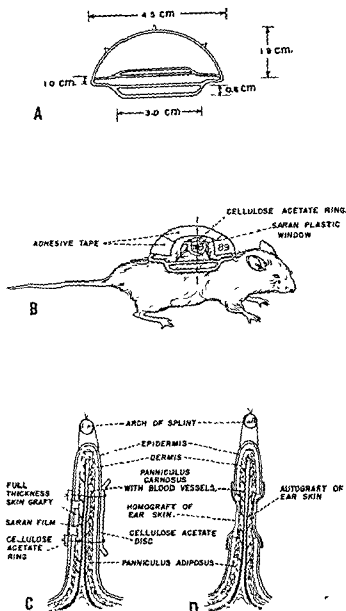


Fig 22 Transparent chamber technique used by Conway, Griffith, Shannon and Findley. A Copper wire traction splint B Usual method of attaching the chamber and splint A fold of the dorsal skin is fixed to the splint. A skin graft is applied to the central part of a bed prepared on one side of this fold, and the whole bed is enclosed in the chamber The two parts of the chamber are held together by sutures C Coronal section through the chamber and skin fold D. Coronal section illustrating an alternative simpler technique in which the chamber consists of two discs of cellulose acetate without the Saran plastic used in the standard chamber This arrangement is optically less satisfactory but permits two grafts to be studied simultaneously. (Reproduced from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Dr Herbert Conway)

scope, mounted on an extension arm, is then swung into place.

Taylor and Lehrfeld (1953) have used this procedure to study the vascularization of skin grafts in rats. With autografts they found that one day after operation many of the vessels of the graft were filled with blood, and these vessels appeared larger than those of normal skin. The blood in the vessels was not clotted. Three days after operation slow circulation could often be seen in the vessels of the graft, which were still distended. After five days the circulation was well established and the calibre of the vessels had returned to normal. It was concluded from the early development of vascularization that anastomoses develop between the vessels of the bed and those of the graft.

The Fluorescein Test

Inspection under ultra-violet illumination after intravenous injection of fluorescein has been used to assess the circulation in superficial tissues (Lange and Boyd, 1942) and in skin flaps (Dingwall and Lord, 1943). The same method may also be used to determine when vascularization occurs in free skin grafts and in free grafts of various tissues in the anterior chamber of the eye (Woodruff, unpublished experiments).

Tissue Induction

In experimental embryology the term tissue induction is used to denote the formative effects exerted by organizers (Chapter 10), and in particular by the primary organizer located in the dorsal lip of the blastopore, on neighbouring cells. The term includes not only changes which occur during normal development but similar changes which may be produced in the vicinity of tissue transplanted from an organizer region to some other site in an embryo or *vice versa*; and it may reasonably be extended, as Levander (1945) has suggested, to include all permanent changes which develop in one tissue—adult as well as embryonic—as a result of its being brought by transplantation into juxtaposition with another. We shall consider two examples of tissue induction in this wider sense.

Pigment Spread After Skin Grafting

It was shown independently by Carnot and Deflandres (1896a, b) and by Leo Loeb (1897) that if black skin from a spotted guinea pig is transplanted to a white area *in the same animal* the white skin around the graft blackens radially outward; conversely, white skin transplanted to a black area in the same animal blackens from the periphery inward. A similar phenomenon occurs in cattle, spotted pigs (Billingham and Medawar, 1950b) and sheep (Hardy, Fraser and Short, 1952), but not in rabbits and mice.

Pigment spread was investigated by Barker (1947a) and others but its true nature was not understood until it was revealed by the elegant experiments of Billingham and Medawar (1948a, 1950b). These experiments are of such fundamental importance that they will be considered in detail; first, however, we must consider some anatomical facts which are of consequence for an understanding of the problem.

In the guinea pig pigmented epidermis, including epidermis from red and chocolate coloured skin as well as from black skin, contains two types of cell: ordinary epidermal cells and pigmentary dendritic cells (sometimes called melanophores). The pigmentary dendritic cells are located in the basal layer of the epidermis, and send out branching processes, up to 100 microns in length, each of which weaves horizontally and upwards between the ordinary epidermal cells and ends in a cup-shaped button intimately applied to an epidermal cell or to the body of a neighbouring dendritic cell (Billingham and Medawar, 1947; Billingham, 1948). Pigmentary dendritic cells form melanin and are dopa-positive (Laidlaw and

. 1952 the ordinary epidermal
other hand, though they may
not manufacture it and
The white skin of a spot
completely dopa negative
1948 has shown by a gold
technique that it contains
dendritic cells. The epi
guinea pig's tongue, on the
ins no dendritic cells
pigment from black grafts in
is noticed three to four weeks
and goes on progressively at
per week slowing later to
of this rate and ceasing
It may be initiated by
epidermis as well as by con-
Pigment spread from
skin takes longer to start
slowly than spread from
to red graft from sur-
still more slowly,
graft in a red area
[illegible]
rather than ho-
uniformly reticulate;
as dense at the
of the darkened
grafts which have
completely blackened,
only black den-
area of the graft
cells in the central
are red and
to be mixed.

Billingham and Medawar discuss four hypotheses concerning the nature of pigment spread.

The first, which may be called the hypothesis of successive cellular replacement, was propounded by Loeb (1915). It asserts that when pigmented and white skin are juxtaposed the pigmented epidermis invades and replaces white epidermis. This can easily be disproved by observing grafts which, in addition to being pigmented, differ from the skin at the site to which they are transplanted in respect of some other character which persists unchanged, so that graft epidermis can be distinguished at all times from host epidermis.

According to the second or diffusion hypothesis some enzyme, co-enzyme or metabolic intermediary necessary for pigment formation, which is absent from normal white skin, diffuses from black skin and so endows the surrounding white skin with pigmentary function. Alternatively, white skin is capable of manufacturing pigment but is normally prevented from doing so by an inhibitor, what diffuses from pigmented skin to white is an anti-inhibitor. These hypotheses are refuted because, as Billingham and Medawar have shown, the descendants of a cell once blackened *continue to* produce pigment, and this function is serially transmissible without loss. It seems clear, therefore, that "whatever it is that causes white skin to blacken must multiply with, or must be repeatedly generated anew in, the tissue that houses it" (Billingham and Medawar (1948b).

According to the third or melanophore migration hypothesis pigmented dendritic cells migrate from pigmented skin to white. This has not been entirely disproved but several considerations make it appear highly improbable. In the first place it is pertinent to ask what happens to the non-pigmentary dendritic cells already in residence. There is no room for both pigmentary and non-pigmentary cells, and, as already mentioned, when red skin becomes blackened there is no mixing of red and black dendritic cells. Secondly, Billingham and Medawar have shown that pigmentary dendritic cells do not migrate away from the ordinary epidermal cells when pigmented skin is transplanted to subcutaneous pockets, the brain, or the mucosa of the tongue. A third piece of evidence concerns the effect of homotransplants of epidermal cells in suspension and will be considered in a later chapter (p. 15).

The fourth hypothesis, propounded by Billingham and Medawar and called by them the hypothesis of an infective cellular transformation, runs as follows When black and white skin are juxtaposed black dendritic cells establish the same sort of cytoplasmic connection with white dendritic cells as blacks do with other blacks and whites do with other whites Some cytoplasmic ingredient of the black dendritic cell then enters its white neighbour, by the same process as that which allows melanin granules to pass from the branch of a dendritic cell into the cytoplasm of an ordinary epidermal cell Once blackened a white dendritic cell remains pigmentary in function

Though it is stated for black skin the hypothesis requires only slight verbal alteration to apply to all pigmented skin It accounts for all the facts so far presented As a critical test Billingham and Medawar transplanted tongue epithelium to areas of black skin and black skin to the tongue In neither case did pigment spread occur, a fact readily explicable by the hypothesis since tongue epithelium as we have seen is devoid of dendritic cells

The cellular transformation is formally equivalent to the spread of a virus infection, but its precise nature has not been fully elucidated It is suggested, however that a melanogenic enzyme system or enzyme precursor, capable of reproducing itself in the cytoplasm of black dendritic cells enters a white dendritic cell and continues to reproduce itself in this cell and in its lineal descendants

Infective cellular transformation may well have a much greater biological significance than is at present realized, and in particular it may play an important part in the propagation of neoplastic disease (Billingham and Medawar, 1948b) It seems likely, moreover, that transplantation will prove to be an important instrument for the further elucidation of this phenomenon

Ossification by Induction

Neuhof (1917) showed in dogs that if a free autotransplant of fascia is used to repair a defect in the urinary bladder, bone develops on the inner (vesical) surface of the transplant The ossification is not due, as Phemister (1923) suggested, to the action of urine on the transplant because it still occurs if, prior to transplantation, the urine is completely diverted from the bladder by establishing bilateral ureterocutaneous fistulae (Huggins, 1931) Conversely, as Huggins and his colleagues have shown (Huggins, 1930, 1931, Huggins McCarroll and Blocksom, 1936), if epithelium of the urinary bladder, ureter or renal pelvis of a dog rat or guinea pig is transplanted autologously to the muscles or fascia of the abdominal wall, cysts develop and bone is formed in the vicinity of the epithelium The bone is typical lamellar bone with haversian systems, and contains haemopoietic marrow In the dog it appears first about 19 days after transplantation, reaches its maximum development after about two months, and thereafter shows little or no change Autotransplants of small portions of kidney, prostate, adrenal stomach, small bowel, colon and dura mater do not cause bone formation Ossification may occur, however, as Nakamoto (1927) was the first to show, in the vicinity of transplants of gall bladder epithelium and also in fascia from the rectus sheath transplanted to the gall bladder (Huggins and Sammett, 1933)

It appears that to stimulate ossification the epithelium must be actively proliferating if, for example, portion of the wall of the bladder down to but not including the mucosa is excised and replaced by a fascial transplant ossification does not occur

A discovery of great interest is that only some of the connective tissues of the body can be stimulated to form bone Thus, for example, in dogs if epithelium from the urinary bladder is transplanted to the interior of the spleen ossification does not

normally occur, but it does so if a piece of connective tissue from the rectus sheath is transplanted *along* with the *epithelium* (Huggins, McCarroll and Blocksom, 1936)

The question of whether ossification occurs in soft tissue surrounding transplants of [illegible] itself is still unsettled The evidence [illegible] is discussed in Chapter 18

Creeping Substitution

[illegible] grafts of bone, unless small, undergo at least partial avascular necrosis With [illegible] transplants the dead bone may be replaced from two sources regenerating host bone and surviving osteogenic tissue [illegible] transplant itself

Replacement by host bone is not peculiar to [illegible] grafts but, as we shall see later, occurs also [illegible] *homografts and heterografts.* The process is discussed in detail in Chapter 18 The graft appears to act mainly as a scaffold- [illegible] has been supposed in addition to be [illegible] local depot of calcium salts. [illegible] unlikely, however, *in view* of the [illegible] Huggins, McCarroll and [illegible] that bone which has been [illegible] strong alkali to destroy organic elements does not stimulate bone formation when used *to fill defects in the skull and ribs.*

Partial replacement of a somewhat different kind may occur with nerve autografts used to bridge defects in peripheral nerves. The graft *shows changes similar to those* which occur in the distal part of a nerve which has been divided and sutured, including proliferation of Schwann cells, break-up of myelin and accumulation of macrophages, *and is* "re-innervated" by regenerating axon fibres growing into it from the proximal part of the nerve (Chapter 17).

Partial replacement by regenerating host tissue also occurs in blood vessel grafts used *to replace* defects in arteries. It is, however, less characteristic of living autografts than of other types of graft (*see* Chapter 21).

The above instances all refer to orthotopic *transplants. Occasionally, however,* a heterotopic transplant may provide a suitable scaffolding for the growth of host tissue, for example, as Javid (1953) has shown in dogs, if a transplant of aorta is used to bridge *a defect in* the oesophagus, *stratified* squamous epithelium grows in at each end and soon lines the whole transplant.

THE FUNCTIONAL ACTIVITY OF AUTOTRANSPLANTS

In this section we shall consider some *examples illustrating factors* which *affect* the functional activity of autotransplants The behaviour of particular tissues and organs will, however, be considered in greater *detail* in later chapters

Skin, Bone, Blood Vessel and Nerve

Skin grafts may *survive* in various sites and may show continued secretory activity, but only *those* used *to* repair surface defects can be said to fulfil the normal function of the skin. Buried grafts commonly form cysts but may be prevented from doing so if *stretched* out and anchored by sutures; they may then be clinically useful but they are *functioning* not as skin *but as supporting* tissue. Similarly, transplants of bone, blood vessel, or nerve, cannot fulfil their normal function unless they form an integral part of *the skeleton*, the vascular tree or *the nervous system respectively.*

Transplants of bone may show structural changes reflecting the functional demands made upon them (Fig. 23). This is a special case of Wolff's law *of* bone transformation (Wolff, 1892; Keith, 1918), according to which every change in the function of a bone is followed by corresponding changes in its internal architecture and its external

form or as Murphy (1918) has expressed it the amount of growth in a bone depends upon the need for it. Functional demand is not the only factor which determines the form of the skeleton for as Fell and Robison (1929) have shown the femur of a five day old chick embryo grown in tissue culture undergoes changes in form similar to those which occur during normal development but it is certainly important.

Transplants of blood vessel and skin may also show changes when subjected to unusual sites: a piece of vein used to bridge a gap in an artery for example becomes thick walled due to increased formation of fibrous tissue.

Endocrine Tissues and Gonads

It might be expected that endocrine transplants would function normally in any site in which they were able to survive. This expectation is only partly fulfilled.

Thyroid autotransplants have been shown to function in subcutaneous and intramuscular sites and in the anterior chamber of the eye (p 184 186). There have been conflicting views about the function of transplants with venous drainage to the hepatic portal system (p 185) but it seems likely that their secretion is inactivated to some extent.

Adrenal autotransplants appear to be functionally effective even when their venous drainage is to the portal system (p 179). Transplants in appropriate sites for example the seminal vesicle of the castrated male or the uterus of the spayed female may produce local androgenic or oestrogenic effects (p 180).

Transplants of the anterior lobe of the pituitary may survive in many sites but do not function normally at any rate in rats except when placed under the median eminence of the tuber cinereum the reason being that only in this position do they become re connected with the hypophyseal portal vessels (p 475).

Transplants of ovary to the tip of the tail

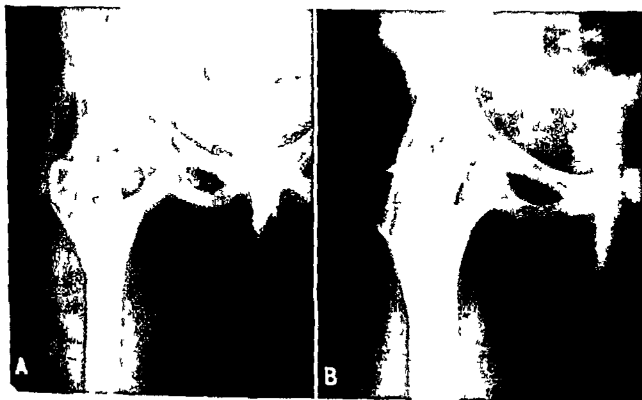


Fig. 23. Radiographs illustrating hypertrophy of a bone graft used in extra articular arthrodesis of the hip. A Before weight bearing allowed. B Five years later. (Mr J. A. Cholmeley's case.)

[illegible] their ability to transform fol- [illegible] corpora lutea, secrete a dimin- [illegible] of oestrogen and also begin [illegible] androgens. this phenomenon has [illegible] uted to an inadequate blood [illegible] is conceivable that low envi- [illegible] temperature may be a factor [illegible]

[illegible] plants of ovary or testis made to the [illegible] or elsewhere in the hepatic portal [illegible] are not functionally effective be- [illegible] their secretions are rendered ineffec- [illegible] passage through the liver [illegible] 1961 [illegible] become, however, extremely active, and often neoplastic, as a result of contin- [illegible] excessive secretion of pituitary gonado- [illegible] Similarly, if one testis of a rat is removed and the other fixed on the abdominal wall, the tubules of the transplanted testis degenerate, but the interstitial (Leydig) cells increase in number and size, and after many months a neoplasm may develop (Bielschowsky and Hall, 1954).

Endometrium

Endometrium may be transplanted deliberately, or spontaneously as in the condition known as endometriosis. In both cases surviving transplants react to hormonal stimuli and show oestrous (or menstrual) changes. This may be readily observed, as Schochet (1929) has shown, in experimental transplants made to the anterior chamber of the eye.

CHAPTER 3

The Typical Behaviour of Homotransplants

Homotransplants as a rule closely resemble autotransplants for a short time, but thereafter, with certain exceptions which will be discussed in the next chapter, the cells of the transplant all die Usually the *whole transplant becomes necrotic and is* either absorbed cast off, or gradually replaced by scar tissue, under certain conditions however, a process of creeping substitution (p 22) occurs

The destruction of a homotransplant may be accompanied by systemic manifestations of various kinds and various degrees of intensity

The rule that homotransplants are destroyed *holds good not only for free homo*transplants but also for transplants by vascular anastomosis and parabiotic transplants, and we shall consider one or more examples in each of these categories

FREE HOMOTRANSPLANTS

The Local Reaction and Its Consequences

The destruction of a homotransplant is accompanied by an inflammatory reaction, often of a violent character This reaction was first described in detail by Leo Loeb and his colleagues (*see* Loeb, 1915 for review), who studied free homotransplants of thyroid, fat, muscle kidney and other tissues, principally in rats and guinea pigs Some of their conclusions have since been disproved (p 70) but their observations have in the main been confirmed

In experiments of this kind the histological findings are often complicated by vascular necrosis of the central part of the transplant similar to that which occurs in autotransplants under the same conditions, the main features of the reaction may nevertheless be easily recognized These are engorgement and rupture of small blood vessels and invasion of the transplant, first by small round cells (Fig 21) sometimes accompanied by polymorphonuclear leucocytes, and later by macrophages and fibroblasts

The intensity of the reaction must be judged by its effect on the transplant It is usually greatest when there is a gross genetic difference between donor and host, but unexplained variations sometimes occur (Woodruff and Woodruff, 1950) The round cell accumulation may be diffuse throughout the whole graft or may be focal in pattern (Woodruff and Boswell, 1953), and its extent is not a reliable index of the intensity of the reaction as a whole The round cells are commonly referred to as lymphocytes, but Darcy (1952), in a study of the reaction to homotransplants of salivary gland tissue in rabbits, identified both lymphocytes and plasma cells

To see the homotransplant reaction in its purest form it is necessary to use transplants of a kind which, if autologous, would sur-

[illegible]

[illegible]

[illegible] and Schultz [illegible] analysis and determine [illegible] population.

[illegible] large punch grafts [illegible] diameter and [illegible] placed in [illegible] after four [illegible] to be [illegible] epithelium [illegible] which surrounds [illegible] the same [illegible] the epithelium and [illegible] the epithelium over the [illegible] and the surface became [illegible] converted to [illegible] undermined [illegible] epithelium [illegible] appear to be in the centre of a [illegible] and subsequently [illegible]

[illegible] Fig. 17 shows that [illegible] of new collagen and epithelial migration, and hyperplasia [illegible] and engorgement [illegible] epidermis and widespread [illegible] the epithelium [illegible] experiments [illegible] grafts from [illegible]



Fig 25 The evolution of skin homografts in the rabbit A Three days after grafting The graft is indistinguishable from an autograft B Six days after grafting The epithelium is still well preserved but round cells have begun to accumulate in the graft C Nine days after grafting The graft has undergone necrosis Most of the surface epithelium has been shed and the dermis is becoming infiltrated by granulation tissue from the graft bed Haematoxylin and eosin × 100

to laboratory bred albinos a striking feature of the histological picture at this stage was the presence of numerous eosinophils in the granulation tissue. This was not observed in Medawar's experiments in which both donors and hosts were drawn from ordinary laboratory stocks, but a similar finding has been reported by Rogers and his colleagues with human skin homografts (Rogers, Converse, Taylor and Campbell, 1953).

The median survival time of homografts between rabbits chosen at random from members of a heterogeneous stock in Medawar's experiments was 10.4 ± 1.1 days

In guinea pigs the inflammatory reaction is less violent than in rabbits and the period of survival of grafts made between animals of different coat colour drawn from ordinary laboratory stocks is remarkably variable (Sparrow, 1953; Woodruff and Boswell, 1954). In Sparrow's experiments, for example, the survival period under standard conditions varied from 5 to 17 days.

In mice the median survival time for full thickness skin homografts of standard size was determined by Billingham, Brent and Medawar (1954a) for different strain combinations of donor and host, and found to range from 8.5 ± 0.2 days for transplants from mice of strain WU to members of a CBA subline, up to 11.0 ± 0.3 days for transplants from mice of the CBA strain to mice of strain A.

In rats Woodruff and Simpson (1955a) found that the survival time of large (2 × 2 cm) split skin homografts from hooded donors to albino recipients was nearly always 13-14 days. The two colonies of rats were non uniform, and their origin is unknown. In more recent observations using hooded rats of a closely inbred strain as donors and albinos of an inbred strain as recipients, the mean survival time has been 10 days (Woodruff, unpublished).

In cattle Anderson, Billingham, Lampkin and Medawar (1951) found that the majority of skin homografts transplanted between un-

[illegible]

Heterotopic bone homografts are gradually absorbed, but since this commonly occurs also with heterotopic autografts care is required in interpreting the phenomena. Orthotopic homografts can be used successfully in both animals and man to fill defects in bones, though healing occurs more slowly than with autografts. As long ago as 1913 Borst suggested that such grafts do not survive but are gradually replaced by bone of host origin, and thereby started a controversy which has not yet been entirely resolved. The uncertainty has arisen partly because the point at issue has not always been precisely defined, and partly because it is difficult to devise really crucial experiments to settle the matter. Today, however, most investigators would agree that, whatever else happens, the cells of a bone homograft do not survive, and all the cells which are present when healing is complete are therefore of host origin.

Orthotopic homografts of blood vessel segments behave in much the same way as orthotopic bone homografts. As we shall see (Chapter 21) they may provide effective long-term, and probably permanent, replacement, and are used regularly in clinical surgery. Carrel, and others of the earlier investigators including Capelle (1908) and Stich and Zoeppritz (1909), believed that such transplants survived permanently. Guthrie (1908c), however, expressed some doubt about this, and his surmise was confirmed by careful histological studies which suggested that, whereas autologous vessel transplants may survive, homologous ones are, as a rule, gradually replaced by host tissue (Borst and Enderlen, 1909a, 1910; Yamanouchi, 1911; Ingebrigtsen, 1912; Myer, 1913).

Later work (Williamson and Mann, 1917; Parsons, Gerbode and Cox, 1952; Benetti and Bellmann, 1953; Eastcott, 1953; and others) has confirmed the view that blood vessel homografts do not survive, but there is still some difference of opinion about the

extent to which replacement occurs and the mechanism of the process. One view which is widely held is that with fresh homografts the endothelium is replaced within a few days, the muscle and connective tissue is replaced more slowly by host connective tissue, and the elastic tissue of the graft persists longest of all. It is probably more correct, however, as Rob (1951d) has pointed out, to say that, while vessel homotransplants are partly replaced by host tissue, they persist to a considerable extent as dead material.

It used to be thought that the new endothelium was formed by ingrowth of host endothelium from the ends of the graft, but it is difficult, on this assumption, to account for the rapidity with which long grafts are re-surfaced. It seems likely, therefore, as Rob (1951a) has suggested, that cells (presumably monocytes) are deposited from the blood stream and assume an endothelial function.

The new connective tissue of host origin which appears in and around an artery homotransplant is, as Bellman and Gothman (1951) and others have shown, richly supplied with blood vessels, and branches of these vessels penetrate into the media of the transplant (Swan, 1952; McCune, Thistlethwaite, Keshishian and Blades, 1952). Kiehn, Benson, Glover and Berg (1952) found the uptake of P^{32} by autografts and homografts was much the same, and that late (30 day old) grafts showed a higher uptake than early (5-10 day old) grafts. They concluded firstly that the process of revascularization is similar in autografts and homografts, and secondly that the nutrition of a re-organized graft depends mainly on the vessels entering the graft from the surrounding tissue and only to a slight extent on diffusion from the blood circulating through the graft.

A point of great interest and importance is that it is not necessary, as far as functional effectiveness is concerned, for either bone or blood vessel grafts to be alive when they are inserted, and good results can be obtained with grafts which have been killed by freezing, freeze-drying, or preservation in chemical solutions of various kinds (Chapter 9). Indeed there is some evidence that dead blood vessel homografts give better results than fresh ones.

Tumour homotransplants often suffer the same fate as homotransplants of normal tissues, but exceptional behaviour is more common. The pattern of the local reaction is not the same for all incompatible tumours, and according to Gorer (1956) three distinct types can be recognized in mice.

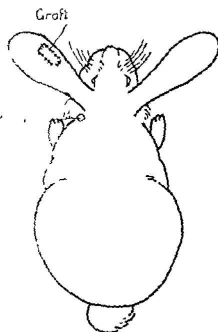
With solid grafts of carcinomas and sarcomas the process of vascularization, round cell infiltration, arrest of circulation and necrosis occur much as with homografts of normal tissues.

Ascites tumours survive in the peritoneal cavity without vascularization, and even when transplanted subcutaneously elicit only a feeble vascular response. After about five days histiocytes may be seen proliferating at the margin of the graft. They advance in a syncytial mass and encircle individual tumour cells. As a rule the tumour cells are killed and phagocytosed, but occasionally the histiocytes die and are phagocytosed by tumour cells.

With leukotic grafts fibrinoid degeneration of collagen and exudation of fluid are usually apparent at the margin of the graft by about the fifth day. The exudation increases and is accompanied by cytolysis of the neoplastic cells. From about the ninth day onwards the graft is invaded by histiocytes, which according to Gorer act mainly as scavengers.

The Reaction in the Regional Lymph Nodes

It has been shown by Scothorne and McGregor (1955) that following homografting of skin to the rabbit ear (Fig. 26) the lymph node draining the area, but *not* the contralateral node or the spleen, *becomes*



[illegible] technique devised by Scothorne and [illegible] studying the reaction in the regional [illegible] homografting (Redrawn from [illegible] York Academy of Sciences by [illegible] and Dr R J Scothorne)

swollen. Histological examination of the affected node shows that the tertiary nodules in the cortex are greatly enlarged, and packed with large lymphoid cells (Fig. 27) which Scothorne (1957) believes originate from reticulum cells. The large-lymphoid-cell response is evident three days after grafting, is fully developed by the fourth day, and remains unchanged in character up to the time of graft breakdown. At 10 days, when the graft is in an advanced state of breakdown, the node is still enlarged but the number of large lymphoid cells is greatly reduced Control experiments have shown that these changes do not occur following autografting.

Craigmyle (1958) has investigated the behaviour of the regional lymph node after homotransplantation of cartilage, again using the rabbit ear as the site for the transplants He observed no changes in the node following a single transplant, but when car-



Fig 27 Section of regional lymph node after homografting of skin, showing two large lymphocytes (A) of the type described by Scothorne The nucleus of a reticulum cell (B) can also be seen but the cytoplasm of this cell is indistinct Unna Pappenheim stain ×1000 (Dr R J Scothorne's specimen)

tilage from the same donor was transplanted to the *same ear on two occasions separated* by an interval of four days the regional node became moderately swollen and showed histological changes *similar to those described* by Scothorne and McGregor. As we shall see in the next chapter this difference in the reaction in the regional lymph node evoked by homografts of skin and cartilage is associated with a difference in the local reactions in the grafts themselves.

Systemic Effects

Lymphocytosis has been reported to occur in recipients of *free homotransplants* by Blumenthal (1939, 1941), and eosinophilia by Rogers, Converse, Taylor and Campbell (1953).

Severe constitutional symptoms have not been observed after a single free homotransplant, or a set of homotransplants made at one operation.

The Significance of Graft Dosage

Medawar (1944) has shown in rabbits that the period of survival of a skin homograft varies inversely with the amount of foreign skin which the host receives. In his experiments the median survival time with 'high dosage" grafts (0.4 g per rabbit) was 10.4 ± 1.1 days, whereas with medium and low dosage grafts (0.05 or 0.006 g per rabbit) the median survival time was 15.6 ± 0.9 days. Medawar found, moreover, that the evolution of lower dosage grafts extended into the period of differentiation characteristic of autograft evolution. Lehrfeld and Taylor (1953), using the method of skin microscopy to determine survival time, found in rats that small grafts 1.4 sq mm in area survived on average 22 days, whereas grafts 1 sq cm or larger survived only eight days.

It must be stressed that in these experiments all the homologous tissue was transplanted *to one site*. It is not known whether the period of survival would be the same if the same total dose of tissue were distributed to several different regions, but Medawar (1951) has suggested that the period of survival might be different if the regions lay in different lymphatic basins, that is to say if they normally drained to different sets of lymph nodes.

What happens if the dosage is made *extremely small*? Does the survival time increase indefinitely or tend to some definite limit? There does not appear to have been any systematic attempt to find the answer to these questions, but Billingham and Medawar (1950b) experimented with very low dosage grafts in their studies on pigment spread in guinea pigs, and found that whereas pigmentation could not be induced by orthodox homografts of pigmented skin it occurred regularly if homologous pigmentary dendritic cells suspended in Ringer's solution were 'seeded' in sufficiently small dosage *on to a recipient area cut to such a* depth that the foreign cells had direct access to the bases of the hair follicles. Moreover, pigmentation once started not only persisted indefinitely in a high proportion of animals but increased in area by the phenomenon of pigment spread (p. 33). At first sight this looks like permanent survival of homologous cells, but Billingham and Medawar have suggested that the phenomenon is due to a serially propagatable transformation of the recipient's dendritic cells from a non-pigmentary to a pigmentary type.

HOMOTRANSPLANTS OF ORGANS BY VASCULAR ANASTOMOSIS

As we have already seen (Chapter 2) the technical problem of transplanting whole organs and limbs by vascular anastomosis was solved during the first decade of the present century, largely owing to the brilliant work of Carrel and Guthrie. As a result many experiments were performed, not only with autotransplants but with homotransplants of limbs, whole organs (including the kidney, spleen, ovary, adrenal gland and thyroid), portions of gut with attached mesentery, and even dogs' heads (see Carrel, 1902, 1906, 1907b, 1908a, b; Carrel and Guthrie, 1906a, b, Guthrie, 1908a).

This work is important because it paved the way for modern investigations, but it throws little light on the biological problem of homotransplantation because the earlier investigators took it for granted that homotransplants would behave similarly to autotransplants, and used both, more or less indiscriminately, to assess the efficacy of various operative procedures. This point of view is well illustrated by the following quotation from one of Guthrie's (1908a) papers:

> "[illegible] have found no evidence of serious d[illegible] of metabolism in dogs' thighs up [illegible] days after transplantation, nor in a do[illegible] foreleg 6 days after transplantation, and [illegible]e there are no physiologic or other reaso[illegible] known why such tissues as those found in the head may not live again and function [illegible] such conditions, it seems justifiable to conclude that it is possible to transplant such a member with permanent success."

In consequence of this assumption in some papers it is not even stated whether the experiments refer to autotransplants or homotransplants, in others the failure of homotransplants is attributed, with little or no apparent justification, to faulty technique (*see e.g.* Carrel and Guthrie, 1906a), and in others again, as the passage quoted above illustrates, conclusions are drawn about permanent survival from experiments in which the period of observation was limited to a week or two, or even a few days.

More recent investigations have established beyond doubt that homotransplants of whole organs by vascular anastomosis, like most free homotransplants, may survive and function for a short time—which differs in different species but in dogs is usually somewhere about a week—but thereafter cease to function and are destroyed. Organ transplantation in experimental animals and man is discussed in detail in Chapters 25 and 26, but it will be convenient to summarize briefly the relevant facts which have now been established concerning the behaviour of homotransplants of one particular organ, the kidney.

In dogs, if the technique is satisfactory, a renal homotransplant begins to secrete urine within an hour, and often within 15 minutes, of re-establishment of the circulation, and continues to do so for a period which varies from 2 to 12 days but is usually about 5 days. At no time however is the function as good as that of an autotransplant of the same age made by the same technique.

Homotransplants examined after three or four days are usually found to have increased in weight, and the pelvis and ureter are swollen. They may or may not be adherent to the surrounding tissue, depending on the site in which they are placed. A little later homotransplants appear bluish in colour, do not bleed freely when incised, and are found to have increased in weight two-fold or even three-fold. The capsule is thickened and strips easily leaving a smooth surface. On section the cortex is wider than normal and has a somewhat nodular appearance. The medulla shows no gross changes other than slight oedema and some blurring of the parenchymal markings.

Histological examination shows that cel-



Fig. 28. Photomicrographs of a kidney homotransplant in a dog after 12 days. A. Haematoxylin and eosin preparation (×250) showed marked accumulation of round cells in the wall of a blood vessel and the surrounding tissue. B. Methyl green pyronine preparation (×250). Note the normal looking glomeruli, the advanced tubular degeneration and the cluster of darkly staining (pyroninophilic) cells in the right hand part of the photographs. (Dr. G. J. Dammin's specimen.)

lular infiltration appears first in the cortex, especially around the glomeruli and small blood vessels, usually on the second or third day Initially the cells are almost all mononuclears though later polymorphs appear. Some of the mononuclear cells appear to be small lymphocytes but many are seen to be pyroninophilic when stained with pyronin methyl green (Unna-Pappenheim stain) and are apparently plasma cells. Mitoses are fairly numerous among the pyroninophilic cells, and Simonsen (1953a) claims that a continuous transition can be discerned between "resting" non-pyroninophilic reticulum cells and "active" pyroninophilic cells, he thus holds that the plasma cells do not come from the host but originate in the transplant, and Dempster (1953a) has arrived at the same conclusion though for different reasons (p 112)

In the later stages (Fig 28) cellular infiltration [illegible] widespread throughout the cortex, and is [illegible] seen in the fat of the sinus renalis and [illegible] the ureter; it is, however, rarely [illegible] the medulla The glomeruli [illegible] ll preserved but may show some [illegible] of the basement membrane. The brush border of the proximal tubules begins to fade about the third day and the distal tubules become dilated; later, droplet degeneration occurs and numerous casts (both hyaline and granular) are to be seen in the tubules.

The renal artery shows no evidence of damage; the endothelium is not swollen nor is it pyroninophilic, and there is no cellular infiltration in the vessel wall. On the other hand the intrarenal vessels, and the vessels of the capsule, sinus renalis fat and ureter, show changes resembling those seen in periarteritis nodosa, and their endothelium is swollen and markedly pyroninophilic.

In the terminal stages the histological picture is one of widespread haemorrhage, oedema and necrosis, more pronounced in the cortex than the medulla but still leaving the glomeruli relatively unscathed.

In man renal homotransplants may function for weeks or even months, but this is not altogether surprising in view of the fact noted earlier that homologous skin grafts usually survive considerably longer in man than in most experimental animals.

PARABIOTIC TRANSPLANTS

Pa[illegible] 1863) introduced the term parab[illegible] denote the procedure of uniting [illegible] of two different animals, in order [illegible] achieved, to establish a common circulation The technique aroused little interest until it was re-investigated by Sauerbruch and Heyde (Sauerbruch and Heyde, 1908 Sauerbruch, 1923) about half a century later, but since then it has been widely used in biological investigations. Highly inbred strains of animals, especially rats and mice, have been used by most workers. Even then the mortality rate is high but not nearly as high as when the partners are chosen at random from a mixed population.

Numerous studies have been made of the behaviour of parabiotic skin grafts, notably by Lund (1908), Finney (1909), Gillies (1935), Manganaro and Faraone (1945) and Moran (1952a) in man, and by Kross (1922), Epstein (1924), Segovia (1924), Rabinovitch (1928), and Monroe, Andreson and Hass (1953) in animals. A more extensive bibliography is given by Rogers (1951).

No case of permanent survival has been reported. Moreover, it has been found that after a few days one of the two individuals usually becomes seriously ill and recovers only when the pedicle is divided Following division of the pedicle the graft undergoes necrosis. Lund, for example, reported the

case of a boy of 19 who received a parabiotic flap from his brother; on the day following operation the brother had a temperature of 103°F., and by the sixth day he was desperately ill though the flap still appeared healthy. The pedicle was divided and it was noted that a little bleeding occurred from the cut end; after a few days, however, the whole flap became gangrenous. In Moran's case the donor developed signs of shock and anaemia four days after operation. Attempts to correct this by transfusion failed, the donor becoming increasingly anaemic and the recipient polycythaemic; the pedicle, therefore, had to be divided. Similar cases have been recorded by Gillies (1935). Rabinovitch (1928) observed the same phenomenon six or seven days after parabiotic skin grafting in the guinea pig. He noted further that, when the animal to become ill was the host, moist gangrene developed in the flap one or two days after division of the pedicle, whereas when the donor became ill the flaps survived for 6-16 days after division and then became dry and shrivelled.

There have been sharp differences of opinion regarding the truth of Bert's postulate that vascular continuity is established across the parabiotic junction. Rabinovitch (1928), Hill (1931), Furth, Barnes and Brower (1940), and Huff, Trautman and Van Dyke (1950) among others have asserted that vascular continuity does occur; Cristea and Denk (1910), Dragstedt and Cooper (1923) and Rogers (1951) have claimed that it does not.

Monroe, Andreson and Hass (1953), after reviewing the experimental findings and considering possible fallacies, concluded that where there was convincing evidence of vascular continuity persisting for long periods of time the parabiotic partners were members of a closely inbred strain. They therefore re-investigated the matter in animals (rabbits) drawn from a mixed population. In view of the importance of the



Fig. 29. Technique of parabiosis in rabbits devised by Monroe, Andreson and Hass. (Reproduced from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Dr. G. M. Hass.)

findings the experiments will be described in detail.

Parabiosis was effected by removing the skin from the lateral aspect of the right ear of one partner and from the left ear of the other, together with the aural cartilage except for a narrow peripheral rim, and then uniting the raw surfaces by suture (Fig. 29). To determine whether there was vascular continuity across the junction one partner was given an intravenous injection of phenolsulphonphthalein and one hour later a catheter specimen of urine was obtained from the other animal and examined for dye, alternatively a solution of Evans blue was injected intravenously into one animal and an hour later the amount of dye in the serum of each was estimated. These tests were repeated at intervals. As a further check, parabiosis was maintained for [illegible] ng periods and at the termination of each experiment Indian ink was injected into the marginal ear vein of one partner at a [illegible] e slightly higher than the arterial [illegible] h base of each ear was then [illegible] and after killing the animals, his[illegible] ions of the junctional region [illegible] examined

[illegible] findings were as follows:

[illegible] e was a high mortality rate, one [illegible] g within 21 days of operation [illegible] t of a total of 63 pairs studied.

[illegible] healing occurred at the junction [illegible] this was followed, after one to two weeks by separation at the line of epithelial union and by necrosis, and lymphocytic and monocytic infiltration, in the junctional zone

3 Vascular continuity across the parabiotic junction was demonstrated in all the pairs tested from the third to the sixth day of parabiosis and in half of the animals tested on the seventh day. After the seventh day vascular continuity could no longer be demonstrated. Angiitis and endarteritis of the vessels adjacent to the junctional zone appeared to play a part in bringing about the loss of vascular continuity.

On the other hand when in control experiments the two ears of a single rabbit were united after removing skin from the medial aspect of each, permanent vascular continuity was established across the junction.

Once vascular continuity becomes established in parabiosis it seems almost inevitable that blood will be transferred from one parabiont to the other since there will almost certainly be an appreciable difference in blood pressure in the two animals. This may be partly responsible for the parabiosis reaction; one would expect, however, that as the blood volume of the weaker partner diminished the blood pressure would fall and the process would be arrested.

Another factor to be considered is that with the mingling of the two circulations incompatible transfusion reactions may occur, if, as in the clinical case described by Manganaro and Faraone (1945), the two partners belong to different blood groups. It must be emphasized, however, that the parabiosis reaction occurs in animals where the blood groups are not easily demonstrable, and also in human beings even when the two partners belong to the same ABO group (Moran, 1952a). It seems unlikely therefore that the phenomenon can be explained wholly in terms of erythrocyte incompatibility. Other possible factors will be discussed later (Chapter 5) when we consider the nature of the reaction to homotransplants in general.

CHAPTER 4

Factors Which May Determine Long Survival of Homotransplants

There are a number of exceptions to the rule that homotransplants are rapidly destroyed The factors which determine long term and in some instances permanent survival will be considered under the following headings

1 Special properties of the tissue comprising the transplant

2 Special properties of the site used for the transplant

3 Compatibility of donor and host due to genetic similarity or other causes

4 Constitutional abnormalities of the host

5 Experimental and therapeutic procedures

SPECIAL PROPERTIES OF CERTAIN TISSUES

Tissues appear to differ in their capacity to survive after homotransplantation To some extent the observed differences depend on other factors such as the quantity of foreign material transplanted or the site in which it is placed this however is not the whole explanation and the matter merits fuller consideration than it has yet received

For the most part the differences are slight but homotransplants of certain tissues have been reported to survive permanently even when the donor and host are genetically dissimilar Some of these claims are well established others however are of doubtful validity and others again are certainly false We shall therefore review the evidence on which they are based

Normal Adult Tissue

Cartilage

As we shall see in Chapter 19 there is abundant evidence that homografts of living cartilage survive commonly for months and sometimes for years, in rats guinea pigs rabbits and dogs There is also evidence that such grafts survive for long periods in man as we shall see however when homologous cartilage is used in reconstructive surgery it is usually dead* and therefore cannot be said to survive though it may under certain conditions persist more or less unchanged for years

As might be expected the long survival of homografts of cartilage in comparison with that of homografts of most other tissues is associated with a difference in the local inflammatory reaction This difference is however one of degree rather than of kind and as Loeb and his colleagues (Loeb 1926a b c 1927b 1915 Loeb and Harter 1926 Loeb and Siebert 1935) have emphasized there is often quite a marked accumulation of small round cells in the neighbourhood of [illegible] homograft which lasts

*[illegible] preserved in an antiseptic solution and [illegible] as well.

for some weeks and is followed by fibrosis and encapsulation Later the chondrocytes may disappear though the intercellular substance remains (Fig 30)



[illegible] ph of part of a subcutaneous [illegible] rabbit after 2 years The [illegible] well preserved but the [illegible] hematoxylin and eosin × 100 [illegible] specimen)

[illegible] p. [illegible]) it seems almost [illegible] ing survival of cartilage [illegible] to the fact that they are [illegible] not readily penetrated by host cells.* [illegible] composition of the ground substance may well be important in this connexion in that it may help to determine the avascularity of cartilage and its resistance to cellular infiltration Bacsich and Wyburn (1947, 1955) have suggested that in addition the mucopolysaccharides of the ground substance exert some more subtle effect, and in support of this view have cited experiments in guinea pigs in which they found that homografts of actively growing cartilage with a high cell content and relatively little ground substance also evoked little reaction and survived more or less unchanged for long periods.

Cornea

As a result of much experimental work homotransplantation of cornea has become an established clinical procedure, but there is no general agreement as to why the operation is so successful.

Cornea, like cartilage, is normally avascular and this would seem at first sight to provide a sufficient explanation. It was observed many years ago by Fleisher (1921), however, that subcutaneous homografts of cornea in guinea pigs were rapidly invaded by host cells and destroyed. It may well be, as Billingham and Boswell (1953) have suggested, that this does not happen with orthotopic grafts because the host's corneal tissue surrounding them is itself avascular. On the other hand it has been suggested that corneal grafts do not in fact survive but are replaced by host tissue by a process of creeping substitution (p. 22)

The evidence for and against those hypotheses will be considered in detail in Chapter 20

Ovary

Much of the vast literature on homotransplantation of ovary is concerned with transplantation between genetically similar (or nearly similar) animals, transplantation from immature donors, or transplantation to special sites such as the anterior chamber of the eye.

In this section, however, we are concerned only with transplantation of ovary from adult donors to genetically dissimilar hosts, using sites which are not especially favour-

*Avascularity *per se* is not enough because, as Billingham and Sparrow (1954) have shown, homotransplants of pure epidermis—which is an avascular tissue—suffer the same fate as ordinary skin grafts What seems to be required is avascularity associated with a graft of such a size and shape that the bulk of the tissue comprising it is effectively insulated from the cells of the host.

able for homotransplants of other tissues Many workers have reported that such transplants survive and function for long periods Much of the evidence is unconvincing because the findings, whether physiological or histological, could be explained by regeneration of the ovarian tissue of the host, but not all the claims can be dismissed in this way

The question is discussed at length in Chapter 21, and here we need consider only the conclusion to be drawn from a critical survey of the literature This is that while ovarian homografts are, as a general rule, *eventually* destroyed, they often survive very much longer than, for example, homografts of skin exchanged between animals showing a similar degree of genetic diversity The reason for this is by no means clear

Tissue from Embryos and Immature Individuals

There have been many reports of long term and even of permanent survival of homotransplants obtained from embryos and immature donors

In many cases there were other factors apart from the immaturity of the donor which may have contributed to, or even been primarily responsible for, the results In some experiments, for instance, the transplants were placed in the anterior chamber of the eye (*e g* Turner, 1938a, 1939, Kammeraad, 1942, Greene, 1943, 1947, Martinovitch, 1949 Dameron, 1950, 1951a, b), or in the brain (Willis, 1935, 1936, 1939), and, as we shall see in the next section (p 55), these sites are especially favourable even for homotransplants of adult tissue In other experiments the donors and hosts were members of the same inbred strain, or the quantity of tissue transplanted was extremely small In others again, as in the experiments with homografts of preputial skin from new born babies described in Chapter 13, several donors were used for one recipient, each donor contributing only a small fraction of the total amount of foreign tissue, and this, for reasons discussed later (p 211), is also *conducive to* long survival

In the absence of these special factors homotransplants of embryonic tissue are less successful

Lexer (1911) found that homografts of human foetal skin took, and enlarged rapidly, but were cast off in the third week Since then claims of long survival have been made, but Baxter and Goldstein (1958a), who used skin from foetuses of 17 to 38 weeks gestation in 8 adults, found that the survival time of the grafts ranged from 1 to 16 days In animals also, as Barker (1947b) has shown, homografts of foetal skin do not survive permanently

Hammond (1927) transplanted embryonic tissues to the uterus of rabbits and found that only cartilage survived as long as 20 days

Willis (1939) found in rats that embryonic cartilage, epidermis, epithelium of mucous membranes and glands of external secretion, striated muscle, and haemopoietic tissue, would survive* and proliferate, not only in the brain but also in subcutaneous tissue and in the peritoneal cavity, after homotransplantation to unrelated adults On the other hand he found that subcutaneous homografts of bone from rat embryos survived no better than subcutaneous homografts of adult bone, and though he obtained a few successful transplants of foetal endocrine tissues he states that his experiments were "too few in number to determine whether embryonic endocrine and gonadal tissues transplant better than their adult counterparts"

The more recent work of Parkes and Smith (Parkes and Smith, 1953; Smith and Parkes, 1954) and Deanesly (1954a, b) does not resolve this question because, although

*In most of Willis experiments the transplants were left in situ for periods varying from 4 to 13 weeks

these investigators obtained a high proportion of long surviving grafts of ovarian and testicular tissue from seven day old albino rats to adult black and white rats, ovarian grafts from older donors were also successful, and testicular grafts from older donors were [illegible] studied

[illegible] with homografts of adrenal [illegible] from foetal and immature animals [illegible] some instances of long sur[illegible] the observations of different [illegible] are conflicting (Chapter 23) [illegible] of human foetal adrenal in [illegible] with Addison's disease have some[illegible] resulted in considerable improvement [illegible] for several months, but in four [illegible] in which biopsy was undertaken 7-10 [illegible] after transplantation (Woodruff, [illegible] no trace of the grafts remained.

[illegible] apparent from the foregoing that [illegible] of embryo tissues do not [illegible] rule survive permanently, but [illegible] sufficient evidence either to [illegible] refute the prevalent belief that [illegible] from embryos and imma[illegible] in general longer than [illegible] corresponding adult tis[illegible] conditions The matter [illegible] by further experiment, [illegible] comparative studies of the [illegible] transplants of tissues from [illegible] individuals of various [illegible] under strictly con[illegible] As will be seen later [illegible] a conclusion which does emerge from the study of transplants in the anterior chamber of the eye is that, while homotransplants of both adult and embryonic tissues may survive permanently in this site, the former rarely if ever grow to more than two or three times their original volume (Woodruff and Woodruff, 1950), whereas the latter may increase up to one thousand fold (Dameron, 1951).

The question arises as to why the mammalian foetus does not share the usual fate of homotransplants. It will, however, be convenient to consider this problem later (p. 187).

Neoplastic Tissue

The maintenance by serial transplantation of malignant tumours which have been experimentally induced, or have occurred spontaneously, in animals is a commonplace laboratory activity.

As a rule closely inbred strains are used, but some tumours can be successfully maintained by homologous transplantation in animals which are by no means homozygous. A striking example is the Brown-Pearce carcinoma—originally a spontaneous epidermoid carcinoma which developed in the scrotal skin of a rabbit—which can be maintained by intratesticular transplantation in rabbits of ordinary laboratory stock. Hauschka and Schultz (1954) have concluded from cytological and genetic studies of mouse tumours that the capacity to grow progressively in foreign strains is associated with aberrations of chromosome number.

An interesting phenomenon has been described by Furth, Boon and Kaliss (1944), who have shown that what they term the *transplantation pattern* is not the same for normal and neoplastic tissues. With two particular pure lines of mice Kaliss and Robertson (1943) found that homotransplants of spleen were successful (meaning apparently that the transplants survived indefinitely) within the line, from pure line animals to F1 hybrids, and from F1 hybrids to related F1 mice, but not from F1 hybrids to the parent line Using the same two lines Furth *et al* found that the transplantation pattern with tumours was quite different, and also differed somewhat for different tumours. Thus tumours induced in either line were transplantable not only within the line and to F1 hybrids, but sometimes also to the other line; and tumours induced in hybrids were transplantable not only to hybrids but sometimes also to one or both

of the parent lines Tumours which arose spontaneously in either line were usually transplantable only within the line and to F1 hybrids, but tumours arising spontaneously in hybrids besides being usually transplantable to hybrids, could sometimes be transplanted successfully to one or other of the parent lines

TRANSPLANTATION TO SPECIAL SITES

Certain sites, especially the anterior chamber of the eye and the brain, appear to be peculiarly favourable for the survival of homotransplants

The Anterior Chamber of the Eye

Transplantation to the anterior chamber* was first performed by van Dooremaal (1873), and a little later Zahn (1884) reported successful transplantation of both homologous and heterologous foetal cartilage to the eyes of rabbits More recently Greene and his associates (Greene, 1938, 1941a, b 1942a, 1943, 1946, 1947, 1949, Greene and Lund, 1944 Greene and Murphy, 1945, Shrigley, Greene and Duran Reynals 1945) have confirmed and greatly extended these observations

Transplants of Embryonic Tissues

Greene (1943) successfully transplanted a variety of embryonic tissues in rabbits, but failed with embryonic endocrine tissue and liver In later experiments (Greene, 1947) he found that in mice embryonic endocrine tissues would survive and grow after homologous transplantation, but again homotransplants of liver were unsuccessful

Browning† (1949) studied isotransplants in an inbred strain of mice, and also homotransplants between mice of two different strains He used a wide variety of tissues from 13 and 16 day embryos including skin, nervous tissue submaxillary gland, thymus lung muscle, spleen, liver, stomach and ileum With a few exceptions both isotransplants and homotransplants increased in size for three or four weeks and underwent differentiation to a more adult type of structure thereafter the isotransplants persisted without further change but the homotransplants regressed and were eventually absorbed In later experiments Browning (1950) transplanted foetal pancreas He found that isotransplants of islet tissue survived in both normal and alloxan-diabetic hosts, but homotransplants were destroyed It will be seen that Browning's findings with homotransplants differ from those of Greene This may be due partly to the fact that they used different species of animals, but another factor to be considered (*v infra*) is that Browning made transplants to both eyes simultaneously whereas Greene used only one eye in each recipient

Dameron (1950a b, 1951) transplanted homologous foetal ovary, testis, adrenal cortex and thyroid in rabbits and guinea pigs Reckoning as successful only transplants which increased in size, became vascularized showed histological evidence of differentiation towards an adult type of structure, and survived for at least 30 days the proportion of successful transplants in a total of over 100 operations ranged from 36 per cent to 60 per cent in intact recipients and from 76 per cent to 95 per cent in recipients made totally deficient in the endocrine in question prior to transplantation Many of the transplants were observed for much longer periods than 30 days and showed no evidence of regression With adrenal trans

* The technique of transplantation to this site is described on p 13 See also Martinovitch (1951b)

† The terminology used throughout this book has been altered from in describing Browning's experiments His own terminology and in particular his use of the terms homotransplant and heterotransplant is somewhat different

to compare this finding with the observation of Merwin and Hill (1954) that subcutaneous transplants of thyroid, made by the transparent chamber technique (p. 31), survived if placed close to the window of the chamber where they remained avascular, but were destroyed within 12 days if they became vascularized.

It seems clear that the brain is not as favourable a site for homotransplants as the anterior chamber. It is curious that vascularization should be incompatible with the survival of intracerebral homotransplants of both guinea pig thyroid and rabbit skin, intra-ocular homotransplants of rabbit skin, and subcutaneous homotransplants of mouse thyroid, yet compatible with the survival of intracerebral homotransplants of immature rat adrenal, and intra-ocular homotransplants of guinea pig and rabbit thyroid and various other tissues; whatever the explanation, however, the facts appear to be well established.

The Testis

Next to the anterior chamber and the brain the testis has long been regarded as the most favourable site for homotransplantation of tumours (Greene, 1949), and Aron, Marescaux and Petrovic (1957) have reported that in guinea pigs this site is particularly favourable to three homografts of anterior and posterior pituitary, thyroid and kidney.

Martinovitch (1950b) has transplanted a testis from one rat to another by an ingenious technique which he terms "parabiotic intratesticular transplantation." A small incision is made in the tunica albuginea of one testis of the host and all testicular tissue scooped out, the testis to be transplanted is then freed from the scrotum, but left attached to the donor by the spermatic cord, and placed in the space created in the recipient. The two animals are fixed together with adhesive tape. After a week or two the spermatic cord connected to the transplanted testis is divided and the animals are separated.

Martinovitch has suggested that a similar technique might be applied to other organs. Thus to make a parabiotic adrenal transplant one would first remove part of one of the donor's testes, displace the remaining portion upwards and suture one adrenal to it. A week or two later the adrenal would be freed from its normal vascular connexions and transplanted with the testicular remnant to the tunica albuginea of the host, prepared as described previously.

The Kidney

A number of investigators have made homotransplants of ovary to the kidney and have reported long survival in various species (Marshall and Jolly, 1908; Lipschutz, 1929; Kallas, 1929; Pfeiffer, 1934).

In rats intrarenal homotransplants of adrenal glands from new-born donors have also been reported to regenerate and function (Rutishauser and Guye, 1936), but on the other hand, Everett (1949) found that homotransplants of adult adrenal cortical tissue to the kidney of an unrelated host were always quickly destroyed.

It would be of interest to investigate the properties of this site more fully, using homotransplants of a variety of tissues and placing them either in the substance of the kidney or, alternatively, just beneath the renal capsule.

The Cavity of the Uterus

It has been reported that tumour homografts survive more readily in the cavity of the uterus than when transplanted subcutaneously (Mohs and Guyer, 1937; Hall, 1941; Homburger, 1955). This apparently holds good only when the transplantation to the uterus is made through an opening in its wall, and not when the transplant is inserted through the cervical canal; presumably therefore injury to the uterine wall somehow facilitates survival of the graft.

Muscle and Muscle Sheaths

In the author's experience (unpublished) homotransplants to muscle, though eventually destroyed, usually survive longer than similar transplants made strictly subcutaneously. A similar observation with tumour transplants has been reported by Earle (1954b).

Other Sites

It has been suggested by Stone, Owings and Gey (1934a, b) that, other things being equal, a transplant has a better chance of becoming vascularized if it is situated near a large vessel. Gaillard (1951) is of the same opinion, and in making homotransplants of human parathyroid he opens the axillary sheath and places the transplants in close contact with the axillary artery or vein. He claims that in addition to facilitating vascularization of transplants in general, this site is in some way especially favourable to homotransplants, but the evidence he has put forward (p. 191) is unconvincing.

A number of other sites, including the peritoneal cavity, the knee joint, and the lumen of the internal jugular vein, have been investigated by the author (unpublished), but none of them were found to be especially favourable for homotransplants.

COMPATIBILITY OF DONOR AND HOST

Similarity of Genetic Constitution

It has been reported by many observers that transplants exchanged between identical twins behave like autotransplants. This has been shown in the case of human identical twins with skin grafts (Bauer, 1927; Brown, 1937; Schattner, 1944; Converse and Duchet, 1947; McIndoe and Franceschetti, 1950), and also with transplants of the kidney by vascular anastomosis (Merrill, Murray, Harrison and Guild, 1956).

Moreover, transplants exchanged between members of an inbred strain of animals survive longer, and evoke a milder reaction, than those exchanged between animals which are genetically more diverse, and if the strain has been maintained for many generations by strict brother-sister mating they too may be indistinguishable from autotransplants (*see* Loeb, 1945 for review), at any rate if the donor and host are of the same sex, or the donor is female and the host male.

The Eichwald-Silmser Effect

The necessity for the qualification at the end of the last paragraph was first recognized by Eichwald and Silmser (1955), who observed that skin grafts from male donors to females of the same inbred strain of mice (C57BL or A/Jax), and also from males of either strain to (C57BL x A/Jax) F1 females, were often rejected after they appeared to be well established, whereas the corresponding male to male, female to female, and female to male grafts nearly always survived permanently.

Two explanations have been suggested: androgen dependence of male skin, and the presence of a histocompatibility gene (*v. infra*) on the non-pairing segment of the Y-chromosome. A scheme for detailed genetic analysis of the phenomenon has been put forward by McLaren and Michie (1958) based on the observation of Short and Sobey (1957) that it occurs in some strains but not in others, and the further investigations of Eichwald and Silmser (1956).*

*A new discovery often reveals anomalies in existing definitions. An isotransplant is customarily defined as a transplant made from one individual to another isogenic or almost isogenic, with it (p. 4). Strictly speaking only identical twins are isogenic, and the qualification *almost* was introduced in order to include the case where two animals were members of a strain which was so closely inbred that a transplant from one

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Feldman (1958) has used the technique of adoptive immunization (p. 80) to show that in mice a Y-determined antigen is present in lymph nodes, liver and spleen. In his initial experiment he immunized a male C57BL mouse against a homologous C3H tumour and showed that lymph nodes transplanted isologously to normal C57BL mice conferred immunity if the recipient was a male but not if it was a female. With some other strain combinations no such difference was observed, but [illegible] occur if the transplantation of isologous immune lymph node was preceded by transplantation of normal lymph node, liver or spleen from a male of the same strain

The Breeding of Uniform Strains

How many consecutive generations of brother x sister mating are likely to be required to produce animals which are virtually isogenic? Sewall Wright (1949) has stated [illegible] approximately 19 per cent of the [illegible] heterozygous in any generation [illegible] lost in the next generation [illegible] brother x sister mating. From this [illegible] the proportion of heterozygosity after 3, 10 and 20 generations [illegible] 53, 12 and 1·5 per cent [illegible] even if the underlying assumptions [illegible] However, this calculation may [illegible] by failure to adopt strict random [illegible] choosing which pair of a litter [illegible] breeding, or by the development [illegible] mutations. It is important [illegible] that as Haldane (1936) has pointed out, *complete isogenicity is unattainable*. In practice, however, a strain which conforms to the definition of an inbred strain given by the Committee on Standardised Nomenclature for Inbred Strains of Mice (1952) is likely to behave as if it were truly isogenic in transplantation experiments. According to this definition "*a strain shall be regarded as inbred when* it has been mated brother x sister for 20 or more consecutive generations. Parent x offspring matings may be substituted for brother x sister provided that in the case of consecutive parent x offspring matings the mating in each case is to the younger of the two parents."

Histocompatibility Genes

Much important information has been obtained from transplantation experiments using donors and hosts differing in some clearly defined way in their genetic constitution.

In the simplest type of experiment hy*brids are produced* by crossing *animals* (usually mice) of two inbred strains and normal or neoplastic tissue is transplanted from members of the parent strains to hybrids and *vice versa*.

With normal tissues the transplantation pattern is remarkably uniform, F1 hybrids being tolerant* of transplants from either parent strain and from related F1 animals, but neither parent strain being tolerant of transplants from F1 hybrids. Thus Loeb and his colleagues (*see* Loeb, 1945, pp. 107-108 for review), using strains of mice that were inbred but by no means genetically uniform, found that transplants of normal thyroid, cartilage and fat from either parent strain to F1 hybrids, or between related F1 animals, behaved like transplants between

to the other behaved like an autotransplant. In the light of the Eichwald-Silmser effect it might seem logical to exclude from the definition of isotransplant cases in which the donor and host are of different sex, but this would raise other difficulties because, as we have seen, the effect occurs only when the donor is a male, and has so far been observed only in certain strains of mice. It seems best, therefore, *simply to draw attention to the* anomaly without making any change in terminology.

*When a homotransplant behaves like an autotransplant the host may be said to be *completely tolerant* of the transplant, and when a homotransplant encounters an unusually mild reaction, and in consequence survives longer than usual but not permanently, the host may be said to be *partially tolerant*. It is sometimes necessary to qualify these terms and the phenomenon under discussion may be termed *innate tolerance* or *genetically determined tolerance* to distinguish it from the condition of specific immunological tolerance mentioned in the next section and considered in detail in Chapter 7.

members of the same strain, whereas transplants from F1 hybrids to the parent strains evoked a much more intense reaction; Little and Johnson (1922), and Bittner (1936) found that transplants of spleen from inbred Japanese waltzing mice to Japanese white mouse hybrids behaved like autotransplants, whereas transplants from the hybrids to Japanese mice evoked an intense reaction; and Kaliss and Robertson (1943), also working with splenic transplants but using mice of two highly inbred lines (Ak and Rf), obtained results similar to those of Loeb except that F1 hybrids were completely instead of partially tolerant of transplants from members of the parent strains or from related F1 mice

Experiments of the same kind with tumour transplants, initiated by Tyzzer and Little (Tyzzer, 1909, 1916, Little, 1914, Tyzzer and Little, 1916, Little and Tyzzer, 1916) and subsequently extended by Little and Strong (1924), Strong (1926), MacDowell and Richter (1930, 1932), Cloudman (1932), Bittner (1935), Gorer (1937), Strong, Hill, Pfeiffer and Gardner (1938), Schweitzer and Furth (1939), Furth and Barnes (1941), Furth, Boon and Kaliss (1944) and others (for general review *see* Bittner, 1935, Little, 1941, 1951, Snell, 1952a, Law, 1954), have yielded results which are generally similar but, as we have already seen (p 54), decidedly less uniform Thus a tumour which grows progressively in members of an inbred line usually fails to grow progressively in unrelated strains (though it may grow progressively in another subline of the same strain*), but grows in F1 hybrids where one parent is from the line of origin of the tumour, and in a proportion of F2 mice and back-cross mice (F1 × resistant parent) A tumour originating in an F1 hybrid usually, though not invariably, fails to grow in either parent line but grows progressively as a rule in all F1 hybrids and in a proportion of F2 and back-cross mice depending on the particular tumour and the strains which are used A tumour originating in an F2 mouse grows as a rule in all F1 mice, and in a proportion of F2 and back cross mice.

These findings suggest that we may distinguish a special class of dominant genes, termed by Snell (1948) *histocompatibility genes,* which determine the fate of homotransplants of normal tissues and to a large extent of tumours, and that complete tolerance results if, and only if, the transplant possesses no histocompatibility genes which are lacking in the host. The atypical results sometimes obtained with tumour transplants may be attributed largely if not entirely to the fact that some tumours are able to grow progressively in the face of considerable resistance (Gorer, 1947, 1948, 1950), to differences between the genotype of the predominant tumour cells and that of the host resulting from mutations (Strong, 1926, Cloudman, 1932, Furth, Boon and Kaliss, 1944, and many others), and to disturbed genic balance consequent on chromosome polyploidy (Hauschka and Levan, 1953)

The number of histocompatibility loci among mice in general may be estimated from the ratios of resistant to non resistant animals in the F2 and back-cross (to resistant strain) generations in a large number of crosses (Snell, 1948), and appears to be at least 14

Further evidence for the histocompatibility gene hypothesis, and information about the inheritance of histocompatibility genes, have been obtained by studying the behaviour of transplants in sublines of animals differing from the donor strain in respect of only a single gene or a small number of genes Such sublines may be initiated by spontaneous mutation or may be developed

*Furth Boon and Kaliss (1944) found that tumours induced in Ak mice would sometimes grow in Rf mice and vice versa but that tumours arising spontaneously in either of these lines failed to grow in the other

by selective breeding as described by Snell (1948)

Experiments showing evidence of histocompatibility in the presence of some recognizable genetic difference between donor and host have been reported by several investigators Castle and Phillips (1909, 1911, 1913) transplanted ovaries to guinea pigs which differed from the donors in a single genetic characteristic and 3 out of 141 recipients subsequently produced young having the genetic character of the donor animals Robertson (1940, 1942, 1945) performed similar experiments in mice. He used yellow mice of an inbred strain (designated 101 Ay) as donors and transplanted a single ovary from each donor to the ovarian capsule of an agouti litter mate after removing one or both of the host's ovaries His criterion of success, namely the production of yellow offspring when the recipient was subsequently mated with an agouti male, depends on the fact that the yellow colour is inherited as a Mendelian dominant By using this test Robertson obtained evidence of successful transplantation in 12 out of 18 operations when the recipients had both ovaries removed No transplants succeeded in experiments in which the recipients [illegible] single ovary removed, nor in a [illegible] of experiments in which [illegible] transplanted from 101A donors [illegible] of a different strain Medawar and McDonald (personal communication) found that transplants of skin would survive permanently despite a slight genetic difference between donor and host, and Fraser and Clayton (1951) have reported permanent survival of skin transplanted in both directions between normal CBA mice and pink-eyed mutants of the same strain which differed from the stock line in respect of a single gene.

The converse type of experiment in which donors and hosts differ in respect of histocompatibility genes only was initiated by Snell (1948), who succeeded in developing by selective breeding what he has called isogenic resistant (I.R.) sublines of mice, i.e. sublines resistant to transplants from the original strain but not distinguishable in any other way. Many such sublines have now been developed, one at least (Borges and Kvedgar, 1952) differing from the original strain by only a single histocompatibility gene. I.R. lines were developed for, and have been used in, experiments with transplantable tumours; it would be of interest, however, to perform similar experiments with transplants of normal tissues.

In yet another type of experiment the donors and hosts differ in respect of linked genes, one of them a histocompatibility gene and the other not. The first observations of this kind were made by Gorer, Lyman and Snell (1948), who found that one histocompatibility locus in mice (designated H-2) is closely associated with the genes fused tail (Fu), kinky tail (Ki) and short tail (T), which are themselves all closely linked This linkage, by providing a convenient marker, makes it possible to detect new alleles at the H-2 locus, and Snell and Gorer and their colleagues have taken advantage of it in carrying out a large scale genetic analysis (Snell, 1953; Snell, Smith and Gabrielson, 1953; Snell and Borges, 1953, Snell, Russell, Fekete and Smith, 1953; Amos, Gorer and Mikulska, 1955). The method has limitations, however, because the histocompatibility phenotype, as determined by growth or failure to grow of tumour transplants, does not always coincide with the histocompatibility genotype (Snell, Smith and Gabrielson, 1953).

More recently Snell, Counce, Smith, Dube and Kelton (1955) have identified another histocompatibility locus, designated H-3, in two strains of mice (C57BL/10 and B10 LP) which are genetically identical except for a segment of the fifth chromosome carrying this locus and the agouti (A) locus with which it is closely linked.

H 3 is a weaker locus than the H 2 in the sense that tumour homotransplants survive more readily when the donor and host differ at H 3 than when they differ at H 2 (Counce, Smith, Barth and Snell, 1956), and it looked therefore as if analysis would be more difficult. Snell, Wheeler and Aaron (1957) have overcome the difficulty however by means of a new technique which they call the *immunization test*. It is based on the fact that the test tumour is ordinarily almost as lethal to B10 LP mice as to mice of the strain of origin (C57BL/10), but is rarely lethal to B10 LP mice which have previously received homotransplants from either C57BL/10 mice or from mice of any strain which does not differ from C57BL/10 at the H 3 locus.

Yet another locus (H 1) carries a histo [illegible]

(Snell and Kelton, 1953; Winn, Stevens and Snell, 1958). Winn *et al.* were unable to distinguish between these strains by skin grafting, but found that an antiserum prepared in strain C3H K could be used to neutralize a leukaemia transmissible normally in C3H mice.

Histocompatibility genetics has not so far been studied extensively in other species, but a start has been made in goldfish by Hildemann and Owen (1956).

Tolerance Acquired In Utero

Transplantation between twins has yielded one unexpected result of great theoretical importance. Since transplants between monozygotic twins behave like autotransplants, it seemed possible that transplantation might be made the basis of a test for distinguishing between monozygotic and dizygotic twins. Anderson, Billingham, Lampkin and Medawar (1951) investigated this problem in cattle and found to their surprise that transplants between twin calves which because of a difference in sex or other characteristics were clearly dizygotic, also behaved for the most part like autotransplants.

It is now known that this is an instance of the condition known as *specific immunological tolerance* (Chapter 7) and results from mingling of the foetal circulations of the twins during intra-uterine life. The evidence on which this interpretation is based will be considered in detail in Chapter 7. We may note here however that the same phenomenon occurs occasionally in other species, and has been observed in human twins.

Another phenomenon, which is probably also an example of specific immunological tolerance and will be considered in Chapter 7, is the occasional long survival of homografts from mother to offspring.

Unexplained Compatibility

Homotransplants sometimes survive unexpectedly for long periods even when the donor and host are quite unrelated.

In guinea pigs Woodruff and Woodruff (1950) found that free subfascial homotransplants of thyroid in thyroidectomized recipients, though usually rapidly destroyed, occasionally appeared normal months after transplantation, even though the donor and host were unrelated and differed in respect of coat colour.

In man the period of survival of skin homografts commonly ranges from a few days to about four weeks but cases of much longer survival have been reported* (p. 212). While some of these claims are undoubtedly mistaken there is in the author's opinion not sufficient evidence to justify the now prevalent belief that, with the exceptions defined in this chapter, skin homografts *never* survive permanently in man.

In hamsters (*Mesocricetus auratus*) pro-

*These include instances of long surviving grafts from offspring to mother, which appear much more puzzling than the long surviving grafts from mother to offspring mentioned above, and also instances of long survival when the donor and host were unrelated.

longed survival appears to be the rule This was discovered by Adams, Patt and Lutz (1956), who found that skin homografts from agouti to agouti, albino to albino, agouti to albino, and albino to agouti, were usually still present 12 months later and during this period showed no evidence of [illegible]matory reaction. Thereafter [illegible] chronic inflammation began to [illegible] including loss of hair, small haemor-[illegible] flaking of epithelium, and formation [illegible] tissue, and these were more marked when the donor and host were of different [illegible]

It has been suggested that the reason why hamsters accept homografts in this way is that they are genetically uniform, apart from variations such as albinism which are the result of comparatively recent mutation, owing to the fact that all laboratory stocks are derived from a single pair of animals which was introduced into Europe many [illegible] This explanation would hold [illegible] only if the members of the [illegible] were identical and homo-[illegible] respect of all histocompatibility [illegible] which would be fan-[illegible] if the number of such [illegible] order in hamsters as [illegible] laboratory animals.

Further experiments (Adams, 1958) have so far not revealed an Eichwald-Silmser effect (p. 59); that is to say homografts from males to females survive just as long as those from males to males or from females to hosts of either sex. For some unknown reason, however, male agouti skin on albino females loses its pigmentation, whereas female agouti skin on male albinos remains pigmented.

It may be asked whether the members of any given species are subdivisible into tissue groups such that homotransplants made [illegible] can be given at present is that such groups may exist, but that if so their number for every species of mammal which has been investigated, apart from the hamster, and for some non-mammalian vertebrates such as goldfish (Hildemann, 1957a), must be enormous.

If tissue groups do exist do they bear any relation to blood groups based on a study of red-cell antigens? The answer to this question, at least in respect of skin groups in man which alone have been investigated in this connexion, appears to be negative (Woodruff and Allan, 1953); it will, however, be convenient to defer consideration of the evidence until later (p. 213).

CONSTITUTIONAL ABNORMALITIES OF THE HOST

Agammaglobulinaemia and Hypogammaglobulinaemia

It was reported by Good and his colleagues (Good and Varco, 1955a, b; Varco, MacLean, Aust and Good, 1955; Good, Varco Aust and Zak, 1957) that homografts of skin from normal individuals to two children suffering from agammaglobulinaemia* behaved like autografts, and form antibody, and inability to resist infection, which they observed in an 11 year old boy. Subsequently two conditions were recognized: *agammaglobulinaemia*, occurring in children and regarded as a congenital error of protein synthesis, and *acquired hypogammaglobulinaemia*, occurring in adults (for review *see* Good, 1954a, Good and Varco, 1955b) The congenital disease has so far been found only in boys and appears to be transmitted as a sex-linked recessive character.

In routine work the plasma proteins are usually fractionated by paper electrophoresis. More accurate methods have shown that even in the congenital cases there is nearly always some gamma globulin present in the serum, so that strictly speaking both conditions should be called hypogammaglobulinaemia. It is customary

*The name *agammaglobulinaemia* was given by Bruton and his colleagues (Bruton, 1952, Bruton, Apt, Gitlin and Janeway, 1952) to a syndrome characterized by absence of plasma gamma globulin, incapacity to

homografts in an adult suffering from hypogammaglobulinaemia* survived for 16 weeks whereas homografts from agammaglobulinaemic patients to normal recipients behaved like normal homografts i e they took initially but sloughed in 2 3 weeks

In these experiments each patient received a split skin graft and a whole thickness graft from a single healthy donor belonging to a different blood group One of the children was observed for a year and the other for four months The grafts retained their original size during the period of observation and tattoo marks which were made on the full thickness grafts at the time of operation remained visible Biopsy of the older graft revealed no evidence of any inflammatory reaction

It is clear that these children did not react in the normal way to the foreign skin, and it seems probable that the grafts did in fact survive as Good and his colleagues believed As they themselves realized however, when the resistance to a skin homograft is for any reason less than normal the epithelium of the graft may be replaced by host epithelium so gradually that it looks as if the graft has survived The possibility of error is not entirely excluded by tattooing but could be eliminated by choosing a donor and host of the opposite sex and determining cytologically the sex of origin of the epithelium at the graft site It is to be hoped that this technique will be used in future experiments

It has been shown (Good 1954b 1955) that the lymph nodes of patients with agammaglobulinaemia are small and poorly developed and that both the nodes and the bone marrow fail to develop plasma cells in response to antigenic stimulation, and it seems likely that it is this deficiency of plasma cells which determines both the agammaglobulinaemia and the absence of reaction to homologous tissue

Attempts have been made to correct the plasma cell deficiency by homotransplantation of healthy lymphoid tissue Good *et al* (1957) transplanted lymph nodes into an adult with acquired hypogammaglobulinaemia and found that immunological reactivity was restored for two months Nicks (personal communication) transplanted the spleen from a two day old baby to a young man aged 22 with agammaglobulinaemia by vascular anastomosis to the inferior epigastric vessels The patient's condition remained unchanged but this is probably of little significance because it seems likely that the vessels thrombosed

It now appears, for reasons which will be discussed later (p 125) that transplantation of immunologically competent tissue to patients incapable of a normal homograft reaction may be dangerous Experiments of this kind have therefore been abandoned until the risk can be more accurately assessed and serious danger eliminated

Uraemia

It has been found (Chapter 25) that, if the operation is technically successful, the period of survival of kidney homotransplants in man varies from two or three weeks to several months Analysis of the reported cases points to the conclusion that survival of the transplant is likely to be prolonged when the recipient at the time of operation is suffering from chronic uraemia

In view of this Dammin, Couch and Murray (1957) investigated the behaviour of skin homografts in six uraemic patients The grafts were observed for periods ranging from 32 to 115 days None of them were completely rejected and some resembled autografts throughout the whole period of observation

Pregnancy

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Sparrow (1953) that skin homografts transplanted to female rabbits about the twenty-second day of pregnancy survive 50 to 100 per cent longer than similar grafts to male or non-pregnant female hosts. A possible explanation of this phenomenon will be considered in Chapter 6.

EXPERIMENTAL AND THERAPEUTIC PROCEDURES

[illegible] to modify the behaviour of [illegible] experimentally, or for therapeutic purposes, will be considered in Chapters 6 and 7.

CHAPTER 5

Homograft Immunity

INTRODUCTION

Four hypotheses have been suggested to account for the destruction of homotransplants

According to the first hypothesis homotransplants perish as a result of *athrepsia* that is to say they die because they are unable to obtain from the host a constant supply of substances essential for their survival This hypothesis was introduced by Ehrlich (1906 1908) to account for his failure to obtain further tumours on reinoculating neoplastic cells to mice with established rapidly growing tumours He held that tumours differed in their avidity for food substances and that those which possessed the greatest avidity had the greatest inhibitory effect on subsequent transplants

Ehrlich s interpretation was criticised by many of his contemporaries including Bashford and Russell (1910) and Haaland (1911) Some of this criticism now appears unconvincing but there remains the decisive objection that the concept of avidity takes no account of the fact that the needs of different tumours differ not only quantitatively but qualitatively Apolant (1911a b c) modified Ehrlich s hypothesis and postulated that the fate of tumour transplants in general depends on the capacity of the host to meet the specific nutritional demands of the tumour Schone (1912a b) Borst (1913) and others have suggested that the fate of homotransplants of normal tissue is determined in the same way

With the discovery that tissues may be cultivated *in vitro* in media consisting of saline homologous or heterologous plasma and homologous or heterologous tissue extract the athrepsia hypothesis has been abandoned There are no grounds for reintroducing it indeed as we shall see the case against it is overwhelming It is worth noting however that the evidence from tissue culture studies alone is not absolutely decisive For a transplant—unlike an explant in tissue culture—may have to compete and may conceivably compete unsuccessfully with host tissue for substances which both require

According to the second hypothesis there is an *innate resistance* to homologous tissue mediated by a humoral mechanism akin to the innate resistance to incompatible homologous erythrocytes It was at first supposed that incompatibility of blood and incompatibility of other tissues were merely different manifestations of the same mechanism, and that homotransplants of all tissues would survive permanently if the donor and host belonged to the same blood group (*see* Davis 1917b 1927 Masson 1918 Shawan, 1919 Baldwin 1920 Dyke 1922) As we have seen (Chapter 1) however skin homografts may break down even when the donor and host are indistinguishable in respect of all the red cell antigens so far studied in this connexion Woodruff and Allan 1953) In its [illegible] form therefore the hypothesis [illegible] be rejected on the evidence already [illegible] but in the more general form

in which it is here presented it merits consideration

The third hypothesis is due to Leo Loeb (see Loeb, 1921, 1930, 1937, 1945). Unfortunately, although he did an enormous amount of experimental work, Loeb has [illegible] given a clear and explicit statement [illegible] hypothesis, but it would seem to em[illegible] the [illegible]lowing two postulates

[illegible] all the tissues and organs in a [illegible] organism have in common a chemical [illegible] termed by Loeb the *individuality differential*, which is peculiar to the individual The individuality differential [illegible] also present in the body fluids of the organism

Secondly, two factors are responsible for the destruction of a homotransplant On [illegible] and the individuality differential of the transplant evokes a cellular reaction in the host, and the cells which accumulate [illegible] to the transplant The [illegible] term varies according to the [illegible] genetic difference between [illegible] but the reaction is usually [illegible]mphocytic On the other [illegible]ality differential of the [illegible] on the transplant [illegible] hypothesis homo[illegible] in consequence of [illegible] which develops [illegible]esis is by no means [illegible] originally by oppo[illegible] hypothesis to account [illegible] of tumour transplants [illegible] and Haaland, 1908, Russell, 191[illegible] Bashford, 1913), and soon afterwards extended to include homotransplants of embryonic tissue and of normal adult tissue (Rous, 1910a, Underwood, 1914, Holman, 1924) The view that the hypothesis holds good for homotransplants of normal tissues was strenuously opposed by Leo Loeb and for many years gained little support; recently, however, it has been restored to favour, largely as a result of the work of Medawar and his colleagues.

Some of the earlier writers used the term *immunity to indicate merely that the resistance to a homotransplant increased progressively, without committing themselves to any opinion concerning the nature of the process. Today however the acquired immunity hypothesis implies that homotransplants act as antigens* and evoke the production of antibodies* which act specifically on the cells of the transplant, either injuring them directly or rendering them susceptible to attack by host cells.*

The acquired immunity hypothesis was based initially on the observation that an animal rejects a second homotransplant from the same donor more rapidly than the first. This *second-set phenomenon* (p. 69), as it is now called,† is not in itself conclusive proof that the underlying process is an immunological one, nor does it exclude the possibility that the resistance to a homotransplant is, in part, innate. Additional and weighty evidence in support of the acquired immunity hypothesis has, however, been provided by the discovery that the state of resistance evoked by a homotransplant can be transferred to another host by the process known as *adoptive immunization* (p. 80), by the study of procedures which decrease the resistance to homotransplants (Chapter 6), and by the discovery of *immunological tolerance* (Chapter 7).

The final proof must depend on the demonstration of the antigens and antibodies whose existence is postulated. A good deal is in fact now known about the antigens

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*The reader who is unfamiliar with the terms *antigen* and *antibody* should consult a textbook of immunology such as that of Boyd (1956).

†The term *second set homografts* was used by Medawar (1944, 1945) to denote a group of skin grafts transplanted at one operation to an animal which had previously received one or more grafts from the same or a different donor The accelerated rejection of second-set grafts from the same donor became known as the *second-set phenomenon*, and this term is now commonly used to include also the accelerated destruction of a single second graft.

(p. 93), but the search for antibodies has proved difficult and often disappointing. As we shall see, however, encouraging progress has been made in recent years (p. 84).

THE SECOND-SET PHENOMENON

In discussing the second-set phenomenon we shall consider experiments with successive transplants of similar and dissimilar tissues, both in non-privileged sites and in special sites such as the anterior chamber of the eye, and also the behaviour of solid homotransplants in hosts which have received injections of dissociated cells, and in those which have been (or are) connected to the donor by parabiosis or cross circulation.

SUCCESSIVE SOLID TRANSPLANTS IN NON-PRIVILEGED SITES

Transplants of Similar Tissue

Experiments with Tumours

It was shown long ago by Ehrlich (1906) that tumour transplants often fail to take in animals which have previously received a transplant of the same or another tumour, and this observation has subsequently been confirmed by many workers; the course of a well-established tumour, on the other hand, is not affected by subsequent transplants to the same host (*see* e.g. Schöne, 1906, 1912a, b; Bashford, Murray and Haaland, 1908; Burgess, 1909; Russell, 1912; Tyzzer, 1916; Woglom, 1929; Besredka and Bardach, 1936). According to Russell (1908a, b) and Woglom (1912) the later transplants fail because the host does not furnish them with "the proper blood supply and connective tissue scaffolding." Da Fano (1912) has confirmed the observation that transplants to "immunized" animals fail to acquire a vascular stroma and has also reported that accumulations of lymphocytes, while commonly found in abundance around primary transplants, are not found around transplants in immunized animals.

The histological features of the reaction to second homotransplants of a tumour have been studied in more detail in recent years, and according to Gorer (1956) three patterns may be recognized, corresponding to the three different patterns of primary response (p. 43). With solid transplants of carcinomas and sarcomas the central feature is, as Da Fano stated, failure to acquire a vascular stroma and consequent ischaemic necrosis. With ascites tumours the histological reaction resembles that evoked by primary transplants but many of the histiocytes are probably blood-borne, whereas in the primary response they are nearly all formed locally. With leukotic grafts, as Potter and Findley (1935) pointed out, lysis of the leukaemic cells commonly occurs before the graft is invaded by host leucocytes.

As we have already seen (Chapter 4) transplants survive when the donor and host are members of a closely inbred strain, or more generally when the donor possesses no histocompatibility genes which are absent from the host. Similarly it has been found that animals (mice) of an inbred strain do not become resistant to transplants of spontaneous tumours arising in members of the strain. As Hauschka (1952) has pointed out this renders untenable the postulate of Gross (1945) that "tumour immunity is directed specifically against the immunizing tumour as such, and is not caused by genetic differences between the cells of the host and those of the animal in which the tumour originated," but it is consistent with the view that neoplasms propagated in mixed stocks evoke immunity directed against them not as malignant growths but as cells derived from genetically different individuals (Eisen and Woglom, 1941).

There has been much argument as to

whether immunity can be induced only by living tumour cells. Snell and his colleagues (Snell, Cloudman, Failor and Douglas, 1946; Kaliss and Newton, 1949; Snell, 1952b) appear to have established, however, that the growth of tumour transplants may be inhibited by appropriate prior injections of lyophilized tumour tissue in small dosage.*

There has also been much discussion about the relation between regression of a tumour and the development of immunity. On the one hand it was suggested that a tumour transplant immunizes the host and thus brings about its own destruction; on the other hand that the tumour cells are killed in some unknown way, and that immunity results from the absorption of substances liberated by the dying cells. The matter has been discussed at length by Woglom (1912, 1929), who has presented convincing evidence that with the Jensen rat sarcoma and probably also with other transplantable tumours, the development of resistance precedes regression.

Experiments with Normal Tissues

Free Transplants of Embryo Tissue. Fi-[illegible] ([illegible]), working with rats, and Rous ([illegible]), working with mice, reported that transplants of embryo tissue were less successful in animals which had received similar homotransplants previously.

Free Transplants of Adult Tissue. Experiments with successive transplants of normal adult tissue have yielded results which at one time appeared irreconcilable. Fleisher (1918) reported that the cellular reaction evoked by free homotransplants of kidney tissue in guinea pigs was intensified in hosts which had previously received intraperitoneal injections of minced homologous kidney tissue. Loeb and his colleagues, on the other hand, found in both rats (Loeb, 1918) and guinea pigs (Hesselberg and Loeb, 1918) that if an animal received a homotransplant of thyroid or other tissue, followed 2-12 days later by a homotransplant of similar tissue from a different donor, there was no significant difference between the reactions to the first and second transplants in respect of intensity, histological pattern or time relationships.

On the basis of these experiments Loeb rejected the acquired immunity hypothesis (*see* Loeb, 1945); his findings, however, are not inconsistent with those of Fleisher, or with the hypothesis, if we postulate that the immunity evoked by a homotransplant of normal tissue from one donor is not necessarily effective against transplants from another donor. More recent investigations, as we shall see, provide convincing evidence of correctness of this interpretation.

Underwood (1914) and Holman (1924) described clinical cases in which multiple skin homografts were used.

Underwood's patient, who had sustained extensive deep burns, received grafts from 17 different donors over a period of about three weeks. The last graft of the series failed to take and the earlier grafts began to melt away, at the same time the patient developed haematuria and cardiac irregularity. Underwood attributed these alarming symptoms to anaphylaxis but they might conceivably have resulted from sepsis in areas not grafted or from some of the curious therapeutic measures to which the patient was subjected.

Holman's first patient, a boy aged five who had lost a large area of skin as the result of mechanical trauma, received 150 small deep grafts (Chapter 13) from his mother. These took, and after five days each graft was surrounded by a ring of outgrowing epithelium. Seven days after the first operation 168 more grafts from the mother were applied; these became firmly attached, but after a further 30 days a condition of exfoliative dermatitis developed which involved

*Prior injections of large doses of lyophilized tumour tissue have the opposite effect. This phenomenon will be discussed in detail in Chapter 7.

all the *grafts* and also, according to Holman, the adjacent normal skin. The second patient, a boy aged two who had been severely burned, received small deep grafts in the form of autografts and also homografts from two different donors (D1 and D2). After 16 days all the grafts appeared healthy and further homografts were applied from a third donor (D3). After a further six days the patient received a second set of homografts from D2 but these failed to take properly. Thirty days after the first operation the original homografts from D1 and D2 were beginning to break down, as shown by the disappearance of the new epithelium which surrounded them, but the grafts from D3 appeared healthy and did not begin to break down for a further 13 days. Holman concluded from these findings that "the agency which caused the first grafts to disappear had no effect on the viability of the grafts from a third donor." And he suggested as a generalization that "the destroying agent is specific for each set of grafts . . . each group of grafts develops its own antibody which is responsible for the subsequent disappearance of the new epidermis." More recent clinical experience with skin homografts from multiple donors (p. 244) suggests that this is not quite true and that a graft from one donor may affect to some extent the survival of a subsequent graft from a different donor. This can readily be explained in terms of the immunity hypothesis if we assume that different donors are likely to have at least some specific antigens in common.

Gibson and Medawar (1943) reported another clinical case. Their patient received two sets of small deep skin homografts from the same donor. The first set grafts began to break down after 15 days and were destroyed in 23 days; the second set grafts, applied 15 days after the first set, showed advanced breakdown after eight days.

Subsequently, Medawar (1944, 1945) made a systematic study of the behaviour of skin homografts in rabbits. He found that small single transplants survived longer than large ones, but that if a set of homografts of different sizes was *transplanted at one operation* from a single donor to a single recipient the period of survival was practically identical for all members of the set.* Furthermore, second set homografts transplanted from the same donor 12 days or longer after the first operation, were destroyed much more quickly than the grafts of the first set. Second set homografts from a different donor, however, sometimes survived as long as the original grafts. In these experiments, and *indeed in all the experiments* with skin grafts described in this chapter, the second set grafts were transplanted to new sites, not to sites previously occupied by the original grafts.

Medawar concluded from his observations that the resistance to homografts is dependent on a systemic rather than a local reaction, and is due to the development of immunity in the host. He concluded further that this immunity "does not necessarily extend with equal vigour" to grafts from another donor. In later experiments Medawar (1946a) showed that there is complete suppression of epidermal cell division in grafts in immunized animals; such grafts survive temporarily, however, in a vegetative state, and during this phase mitotic activity can be restored by transplanting the graft back to the original donor. More recently still Medawar (1954) has suggested that the suppression of mitotic activity is nothing but "a secondary consequence of vascular deprivation." This and most other histological differences between first and second set homografts from the same donor are a consequence of the fact that the reaction between graft and host takes place

*The recent observation of Baxter and Goldstein (1956b) that simultaneous skin homografts from a pair of human identical twin foetuses to a single recipient broke down simultaneously is of interest in this connexion.

within first set grafts and does not become manifest until vascularization is well advanced, whereas second-set grafts fail to become vascularized* and undergo ischaemic necrosis, and the inflammatory reaction, such as it is occurs in the graft bed (Fig. 31).

Medawar's findings have been confirmed by many independent observers including Dempster and Lennox (1951) and Allgower, Blocker and Engley (1952). Dempster and Lennox studied in particular the fate of grafts from different donors They found that in rabbits which had received skin homografts from seven different donors some of the grafts appeared healthy when examined 10 days later, whereas in rabbits which had received the same total weight of graft from a single donor all the grafts showed complete destruction of the epidermis when examined after the same interval.

It was at first suggested by Rogers (1950) that the greatly accelerated breakdown of second-set skin homografts is peculiar to rabbits, but later (Rogers, 1954a) he was able to confirm the observation of previous investigators that it occurs also in man. The phenomenon has also been demonstrated in guinea pigs (Sparrow, 1953), mice (Billingham, Brent and Medawar, 1954; and others), rats (Woodruff and Simpson, 1955a), dogs (Thomas, Murray and Couch, 1957) and goldfish (Hildemann, 1958).

In guinea pigs Billingham and Medawar (1950b) made the striking observation that pigmentation which had been induced in albino hosts by homotransplantation of pigmented basal-layer epidermal cells in very small dosage could subsequently be bleached

*There is also no evidence that second set grafts acquire lymphatic connexions

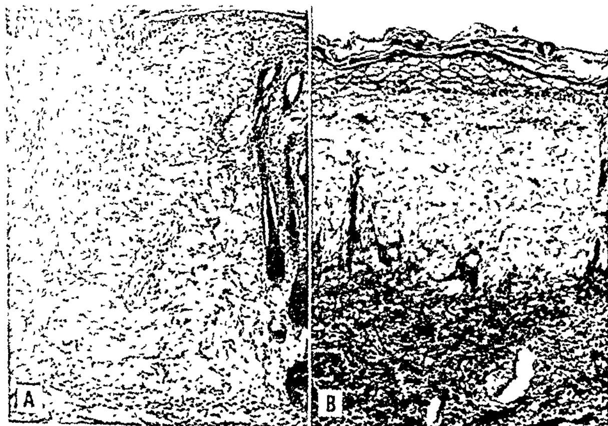


Fig 31 First and second set skin homografts in the rat A First set graft after 7 days. The general structure is well maintained but there is a moderate degree of round cell infiltration in the graft. B Second set graft after 7 days The graft is completely necrotic There is gross accumulation of round cells in the bed but there are few cells in the graft Haematoxylin and eosin ×100.

out by grafting skin in adequate dosage from the same donor

By varying the interval between first and second grafts it should be an easy matter to determine how long it takes for a state of increased resistance to develop following homografting, and how long this state persists subsequently

The first question has not aroused as much interest as it merits In his early experiments on rabbits Medawar (1945) found that second set grafts transplanted from the same donor less than 12 days after the first set behaved like first set grafts This observation is rather surprising in view of the fact that first set grafts had a median survival time of only 10 4 days, and showed evidence of commencing breakdown some days before they were finally destroyed, but there appear to be at least two possible explanations. In the first place it may be that the immunity evoked by a graft is temporarily concealed because antibodies are removed from the circulation by the graft itself Secondly, it is conceivable that the immunity, although widespread, varies in intensity and is maximal in the vicinity* of the transplants It is easy to devise experiments to test these hypotheses but so far there is no record of their having been performed

The second question has been answered as far as skin homografts in mice are concerned by Billingham, Brent and Medawar (1954b), who studied the behaviour of second grafts transplanted from A to CBA mice 15, 30, 60 and 120 days after a first graft from an A line donor The grafts transplanted after an interval of 15 days did not become vascularized and the epithelium was completely destroyed within six days, whereas grafts transplanted after an interval of 120 days became vascularized for a time, showed histological changes more like those seen in a first graft, and sometimes showed surviving epithelium after eight days The behaviour of grafts transplanted after intervals of 30 to 60 days was intermediate between these two extremes It was therefore concluded that the immunity evoked by the first grafts declined after 15 days but was still considerable after 120 days

A second set phenomenon has also been demonstrated in conjunction with the Eichwald Silmser effect (p 59) Thus Eichwald, Silmser and Wheeler (1957) found that the mean survival time of first skin grafts from male C57BL mice to female F1 hybrids was 31 ± 8 days, whereas the mean survival time of second grafts of the same kind was only 13 ± 0 days.

Transplants by Vascular Anastomosis. Dempster (1953a, 1955) studied the behaviour of successive whole kidney homotransplants in greyhounds

In some experiments one host kidney was removed and a homotransplant was made to the neck Several days later when this ceased functioning it was removed After a further 3-7 days the remaining host kidney was removed and a second homotransplant was made to the neck Dempster found that when the two transplants were obtained from different donors the second transplant in 1 out of 7 cases secreted as long as the first and showed similar histological changes When, on the other hand, both transplants were from the same donor the second transplant behaved quite differently It began to secrete, but the flow diminished after 12 hours and usually ceased after 24 hours Second kidneys removed after 24 hours showed gross histological changes including advanced tubular degeneration, widespread interstitial haemorrhage and oedema, and the presence of subcapsular fibrinoid deposit in the glomeruli.

In other experiments Dempster removed the first transplant after 1 4 days while it was still functioning, and made the second from the same donor four days after the first The

*Possibly through the whole of the lymphatic basin in which the transplants are situated

second transplants behaved like normal first transplants, from which it was concluded that there had not been sufficient time for immunity to develop

Simonsen and his colleagues (Simonsen, Buemann, Gammeltoft, Jensen and Jorgensen [illegible] 1953, Simonsen, 1953a) performed somewhat similar experiments in mongrel [illegible] They found that when the first transplant was removed after 3-4 days and re[illegible] by a second transplant from another [illegible] the second transplant behaved like [illegible] normal first transplant, when the second transplant was from the same donor, however [illegible] it functioned for a shorter time than the first transplant, and histological examination after four days revealed changes similar to those observed in second kidneys by Dempster

It seems clear from those experiments that [illegible] property of inducing in the host [illegible] enhanced resistance to subsequent [illegible] from the same donor is not [illegible] transplants but is character[illegible] homotransplants by vascular [illegible]

[illegible]nsplants of Dissimilar Tissues

[illegible] 1953b) studied the fate of [illegible] of skin and kidney in dogs. [illegible] kidneys transplanted to dogs [illegible] previously received two sets [illegible] homografts from the same donor [illegible] with one exception) for only 2-6 [illegible] Conversely, skin homografts on dogs which had previously received a whole-kidney transplant from the same donor were destroyed in about eight days as compared with the normal survival time of 14 days.

Simonsen, Buemann, Gammeltoft, Jensen and Jorgensen (1953), in somewhat similar experiments, found that dogs which had received either a whole spleen homotransplant or free skin homografts subsequently reacted more violently to a kidney transplant from the same donor than to one from another donor

Hardin (1956a) studied the behaviour of whole lung homotransplants in dogs which had received skin grafts from the same donor and *vice versa.* The survival time of first skin grafts to normal dogs ranged from 11 to 14 days, and whole lung homotransplants in normal dogs appeared to function for 5-6 days, but as might be expected the end point was not as sharp as with kidney transplants. Skin grafts made 7-14 days after a previous lung transplant from the same donor (which was left in place for 2-7 days and then removed) survived for only 4-5 days, and lung homotransplants from hosts which had previously received a skin homograft from the same donor functioned for 2-3 days.

These experiments not only help to confirm the acquired immunity hypothesis but point to the conclusion that such diverse tissues and organs as skin, kidney, spleen and lung have transplantation antigens in common.

IMMUNIZATION BY INJECTION OF DISSOCIATED CELLS

Experiments with Tumours

It has been known for many years that an animal may be rendered resistant to homologous tumour transplants by prior injection of homologous living normal cells in the form of defibrinated blood or minced tissue from embryos or adults (*see* e.g. *Schöne, 1906;* Bashford, Murray and Cramer, 1907; Borrel, 1907; Russell, 1908a; Higuchi, 1912; Rous, *1910a, 1913;* Woglom, 1912, 1929). The resistance appears to be maximal about 10 days after injection of blood, and 10-18 days after injection of minced tissue According to Haaland (1910) and Higuchi (1912) injection of dead cells is ineffective

The degree of resistance induced varies with the genetic inter-relationship of the host, the tumour and the donor of the normal cells, and Barrett (1910) found that no

resistance could be induced in a mouse of an inbred strain against a tumour derived from the same strain. The injection of autologous cells has no effect on subsequent tumour transplants (Woglom, 1911); and the injection of homologous cells has no effect on the development of spontaneous or induced tumours (Haaland, 1911; Russell, 1912), nor does it influence the course of an established tumour transplant (*see* Bashford, Murray and Cramer, 1907; Russell, 1912; Woglom, 1929).

Experiments with Normal Tissues

Schöne (1912a, b) found that the destruction of skin homografts in rabbits was accelerated in hosts which had previously been inoculated with homologous embryo cells, and Tschernischoff (1914) also working in rabbits found that the cellular reaction around ovarian homotransplants was intensified in hosts which had been inoculated with minced homologous liver tissue.

Medawar (1946a, b) showed in rabbits that skin homografts were destroyed abnormally quickly in animals previously inoculated with leucocytes from the same donor, but not in animals which had received injections of donor erythrocytes without admixed leucocytes. He reported that the immunizing effect of leucocytes was much greater if they were injected intradermally than if injected intravenously.

More recently it has been found that a high degree of resistance to skin homografts from the same donor can be induced in mice by intravenous or intraperitoneal injection of dissociated spleen cells and peripheral blood leucocytes in adequate dosage, but the resistance reaches its peak much sooner than it does after a solid homograft. Thus, if a mouse is given a skin homograft at the same time as a massive intraperitoneal injection of spleen cells from an animal of the donor strain, the graft is totally destroyed within six days (Medawar, 1959). A curious observation for which as yet there is no satisfactory explanation, is that intravenous injection of blood leucocytes or whole blood will not immunize rabbits (*see*, however, p. 76) although spleen cells are perfectly effective by the intravenous route (Billingham, 1958). This may be related in some way to the observation of Billingham and Sparrow (p. 136) that prior intravenous injection of dissociated epidermal cells will actually prolong the life of skin homografts in rabbits.

HOMOTRANSPLANTS IN ANIMALS JOINED BY CROSS CIRCULATION OR PARABIOSIS

Morpurgo (1914a, b) working with mice, and Kross (1921) with rats, found that an animal of a strain resistant to a particular tumour remained resistant when united by parabiosis to a tumour-susceptible animal of the same species. Furth, Barnes and Brower (1940) found conversely that immunity to a transplantable leukaemia was not transmitted to a parabiotic partner. Gorer (1942) has suggested that this last result is not inconsistent with the immunity hypothesis, because antibodies formed by the resistant animal will be largely absorbed by the normal cells of the parabiont and so fail to reach the transplanted cells in adequate concentration.

The fate of homotransplants of normal tissues interchanged between parabiotic partners has also been studied. Kross (1922) established peritoneal parabiosis in young adult rats and 10 days later transplanted half of a lobe of the thyroid gland from one parabiont to a muscle pocket in the abdominal wall of its partner. He found that the transplants evoked a slightly more violent reaction than, and were destroyed at least as quickly as, similar homotransplants in rats *not joined by parabiosis*. Padgett (1932) found that following the establishment of parabiosis each partner became increasingly resistant to free skin homografts from the

other, and also, rather surprisingly, to skin autografts.

Lang, Dammin and Miller (1955) exteriorized one kidney of a rat, keeping the renal pedicle intact, and buried it subcutaneously in another rat.* The animals were then strapped together for 21 days. There was little or no cellular reaction at the boundary between the kidney and the host, and vascular continuity was not established between [illegible] animals; the procedure was therefore termed *pseudoparabiosis.* In other experiments true parabiosis was established by skin union in addition to renal transplantation by the technique just described, and cellular infiltration, fibrosis and arteri[illegible] then developed in the course of a week in the capsule of the transplanted kidney.

Kamrin and Kamrin (1955) established parabiosis between newly-weaned litter-mate albino rats (of a non-uniform strain). The procedure was successful—as judged by [illegible]lete obliteration of the suture line by [illegible] day of union, equal growth of the [illegible] and equal hair growth with no [illegible]lopecia—in about 25 per cent of [illegible] In this event the adjacent kidneys [illegible]posed and one third of each was [illegible] and reciprocally transplanted as a [illegible] to the defect created in the part[illegible]idney. About half of the grafts formed [illegible] union with the host, and remained viable throughout the period of observation (up to 92 days). When, however, the parabionts were separated prior to reciprocal transplantation of kidney tissue the grafts were destroyed, as were grafts exchanged between control animals that had never been in parabiosis (Kamrin, 1956).

Egdahl and Hume (1957) established cross circulation for periods ranging from five minutes to three days between pairs of mongrel dogs, at flow rates ranging from 250 to 800 c.c. per minute. From 7 to 120 days later one kidney was transplanted by vascular anastomosis from one animal of the pair to the other. It was found that with two exceptions these transplants behaved just like second kidney transplants, and functioned on average for only 1.8 days.*

Egdahl and Hume (1957) showed also that skin homografts exchanged between rabbits which had been cross-circulated for an hour behaved like second grafts.

It thus appears that cross circulation is an efficient means of inducing immunity to homotransplants, even in rabbits, which, as we have seen (p. 75), are not immunized by a single intravenous injection in a dosage which, given intradermally, would produce a high degree of immunity. Parabiotic union also results in immunity which becomes manifest when the animals have been separated, but is not apparent while they remain united.

SPECIAL PROPERTIES OF TRANSPLANTS IN THE ANTERIOR CHAMBER

Experiments with two successive homotransplants, using the anterior chamber of the eye as the site for at least one of the transplants, have yielded conflicting results.

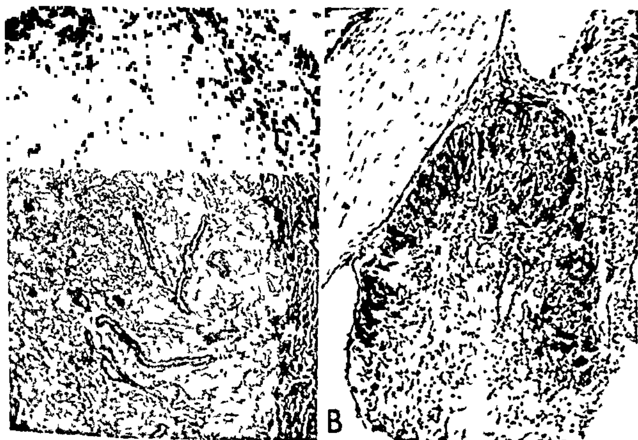
Saphir, Appel and Strauss (1941) found that after a subcutaneous transplant of the Brown-Pearce carcinoma in a rabbit had regressed a second transplant would take and grow in the anterior chamber though not in a subcutaneous site; in consequence, they argued that the state of immunity induced by a subcutaneous transplant does not extend to the anterior chamber. Besredka and Bardach (1936), and Cheever and Morgan (1942), however, found that secondary intraocular transplants of this tumour were usually unsuccessful in hosts which had received a previous transplant to some other site, though successful following a previous

*Donor and host were both albinos but were not members of a genetically uniform strain.

*These investigators found that first kidneys functioned on average for 5.9 days (Hume and Egdahl, 1955) and second kidneys for 1.6 days (Egdahl and Hume, 1956).

transplant to the opposite eye, they therefore concluded that the anterior chamber does share in the state of immunity induced by transplants elsewhere, but that transplants in the anterior chamber do not themselves induce immunity Greene's (1942b) results were different again He found that after homotransplantation of the Brown-Pearce carcinoma to the anterior chamber of one eye both the testicle and the opposite anterior chamber were resistant to further transplants, and concluded that transplants in the anterior chamber do induce immunity and that this immunity extends to the opposite anterior chamber

Woodruff and Woodruff (1950) made somewhat similar experiments with transplants of normal thyroid tissue in guinea pigs drawn from a mixed population As we have already seen (Chapter 1), thyroid homotransplants in thyroidectomized recipients commonly survive permanently in the anterior chamber of the eye, whereas they are usually rapidly destroyed when placed in subcutaneous or subfascial sites It was found, however, that a subcutaneous transplant made at the same time as, or a month preceding a transplant to the anterior chamber from the same donor, significantly reduced the chances of survival of the latter (Fig 32A) Moreover, a homotransplant to one anterior chamber of an intact (non thyroidectomized) recipient—which was slowly absorbed in accordance with Halsted's principle (p 25)—rendered the opposite anterior chamber completely resistant to a



rior chamber of the eye in immunized guinea accumulation of round cells at the periphery of thyroid from the same donor Haematoxylin and eosin ×100 B Section through the anterior chamber showing portion of the cornea and iris, and the remains of a thyroid homograft consisting of fibrous tissue infiltrated with round cells The host had received a previous homograft of thyroid *to the opposite anterior chamber from the same donor* Haematoxylin and eosin ×100

further transplant from the same donor even though thyroidectomy was performed in the meantime (Fig 32B). When, on the other hand, a transplant had become vascularized and had survived in the anterior chamber for six months, a subsequent subcutaneous transplant from the same donor did not have any deleterious effect upon it (Fig. 33A).

Experiments similar to those described, except that the two transplants were taken from different donors, yielded less consistent results, suggesting once again that the resistance induced by a transplant from one donor does not necessarily affect transplants from other donors.

Woodruff and Woodruff showed further that in a significantly high proportion of cases a homotransplant which had been established in the anterior chamber for three to six months could be successfully transferred to a subcutaneous site, and would there become re-vascularized and survive permanently (Fig. 33B).

They also found that when an autotransplant of spleen and a homotransplant of thyroid were placed alongside each other in the anterior chamber the thyroid transplant was soon invaded by round cells and destroyed, whereas homotransplants of spleen and thyroid from the same donor did not appear to exert any deleterious influence on each other.

The following conclusions were drawn from these findings:

Firstly, subcutaneous homotransplants of thyroid in guinea pigs do induce a state of immunity in the host, and the same is true

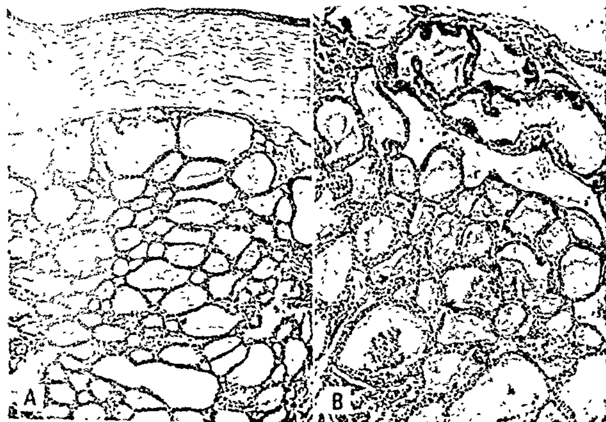


Fig 33 Evidence of adaptation in homografts in the anterior chamber of the eye. A. Thyroid homograft in the anterior chamber of a guinea pig which received a subcutaneous graft from the same donor 23 days after the anterior chamber graft B. Thyroid homograft in a guinea pig 44 days after successful transfer from the anterior chamber to a subcutaneous site.

of thyroid homotransplants in the anterior chamber provided these are undergoing destruction.

Secondly, the state of immunity induced by thyroid homotransplants is general and extends to the anterior chamber of the eye.

Thirdly, the initial period—probably the first few weeks—in the life of a homotransplant in the anterior chamber is critical, in the sense that during this time the transplant is liable to be destroyed as the result of an immunological reaction, whereas subsequently it becomes much less vulnerable.

Fourthly, immunity develops more slowly after an anterior chamber homotransplant than after a subcutaneous one. This was not proved directly but would seem to afford the only explanation of the fact that homotransplants normally survive in the anterior chamber but fail to do so when a subcutaneous transplant of identical tissue is made at the same time. That the rate at which immunity develops should depend on the site of a transplant need occasion no surprise. It is well known that following subcutaneous injection of toxoids and other soluble antigens* immunity is often produced more rapidly than when the same material is injected intravenously (Freund and Bonanto, 1941), apparently because from the former sites much of the antigen is rapidly absorbed via lymphatics and transported to the regional lymph nodes (*see* McMaster and Hudack, 1935; McMaster 1946, 1953). It seems likely that with subcutaneous transplants metabolic products are absorbed directly into lymphatics and cause prompt immunization, whereas with transplants in the anterior chamber these products pass to the aqueous humour and thence via Schlemm's canal directly to the venous system, where they are rapidly diluted and are antigenically less effective.

Fifthly, after a transplant has become established in the anterior chamber, a gradual process of adaptation occurs, as a result of which the transplant may survive subsequent transfer to a subcutaneous site.

Finally, the anterior chamber loses its special properties when it is provided with autologous reticulo-endothelial tissue. It remains to be seen whether this tissue acts simply as a source of cells inimical to a homotransplant, or as a local service of antibodies, or in both capacities.

Perhaps the most important result of these experiments is the discovery that after a time a homotransplant in the anterior chamber becomes invulnerable and is able to survive under conditions which would have proved lethal had the transplant been subjected to them at an earlier stage in its life history. With thyroid transplants the critical period appears to end soon after the transplant becomes vascularized. This contrasts with Medawar's (1948b) observation that homotransplants of skin survive in the anterior chamber, the cornea and the brain only in so far as they fail to become vascularized.

The process of becoming less vulnerable as time goes on had not previously been reported with homotransplants of normal tissue although, as we have seen (p. 75), it has long been known to occur with tumour transplants. The author has suggested (Woodruff 1952a) that the same phenomenon might occur with homotransplants of normal tissue in sites other than the anterior chamber, and that if these could by some means be made to survive for longer than a certain critical period they would survive permanently, and as we shall see (p. 96) there has been some experimental confirmation of this hypothesis.

Some at least of the special properties of grafts in the anterior chamber are shared by orthotopic corneal grafts. Thus it has been shown (Mueller and Maumenee, 1951; Maumenee 1951) that corneal grafts in the rabbit, which normally remain permanently

*Particulate bacterial antigens, on the other hand, appear to be more effective when given intravenously (see F[illegible] 19[illegible]).

clear in 50 per cent of recipients, become cloudy if skin is transplanted from the same donor to the same recipient two weeks after the corneal grafting operation. It seems likely that homotransplantation of skin from the same donor before, or at the same time as the corneal transplantation would have the same effect, though Maumenee did not try this. He did, however, find that transplantation of skin six weeks or more after the corneal transplantation did not cause clouding. Here again, then, we find a critical period. If, as some believe, corneal homotransplants are gradually replaced by host tissue, the inability of a skin graft to cause clouding may indicate merely that the process of replacement is well advanced; if corneal homotransplants do survive, however, the critical period has the same significance in Maumenee's experiments as in those of the author.

SANDERS' PHENOMENON

Darcy and Medawar (1954) found in rabbits that the behaviour of skin homografts was not modified as a result of prior transplantation of tissue killed by freezing and thawing, by treatment with formaldehyde or proflavine, by incubation in etherized serum, or by heating to 50°C.

On the other hand a homotransplant of living tissue may affect the behaviour of a subsequent transplant of dead tissue from the same donor. This was first observed by Sanders (1952, 1954a), who found that frozen-thawed nerve homografts in rabbits, which are normally quickly repopulated with Schwann cells and outgrowing axons (Chapter 17), were rapidly infiltrated with lymphocytes when transplanted to hosts immunized with skin from the same donor.

Subsequently Darcy (1955a, b) compared the behaviour of subcutaneous homotransplants of submaxillary salivary gland tissue which had been killed* by freezing and thawing in normal rabbits and rabbits which had previously received a homotransplant of fresh tissue from the same donor. He found that after 12 days there was a marked difference between the transplants in immunized and non-immunized hosts, the former showing definite leucocytic infiltration and the latter no cellular infiltration except for macrophages and fibroblasts. If however grafts of tissue which had been frozen and thawed were left long enough in normal hosts they too elicited a leucocytic reaction.

It thus appears that repeated freezing and thawing to a large extent destroys the transplantation antigens, but allows the tissue to behave like a hapten† preparation.

*It appears from experiments with autotransplants that the great majority of the cells in the transplants were killed by this procedure but, as Darcy has pointed out, it is not certain that there were no survivors.

†See Boyd (1956) p. 163 for definition of a hapten.

ADOPTIVELY ACQUIRED IMMUNITY

So far attempts to transfer immunity to homotransplants passively from the host to another animal of the same species by serum have failed, except for some comparatively recent experiments with transplantable leukoses in mice which will be considered later (p. 89).

The possibility of transferring immunity by cells was investigated many years ago by Potter, Taylor and MacDowell (*v. infra*), but the far-reaching implications of their findings were not apparent until the work of Landsteiner and Chase (1942) on the transfer of cutaneous sensitivity to simple compounds, and of Chase (1945, 1953) on the transfer of cutaneous sensitivity to tuber-

culin established the principle that some forms of acquired sensitivity can be transferred by cells but not by serum.

As we shall see it has now been proved that *immunity to homotransplants* of both tumours and normal tissues can, under certain conditions, be transferred in this way and for reasons which will be considered later (p. 82) the process has been termed adoptive immunization.

Before reviewing the relevant evidence we may note in passing that the discovery of homograft immunity has provided an explanation for some of the anomalous results which have been obtained in experiments on the cellular transfer of hypersensitivity. If the cells are transplanted isologously no complications arise, but if, as in all the earlier experiments and many recent ones, the cells are transplanted homologously, then the intensity of the homograft reaction which they evoke may have an important influence on the results of the experiment.

Experiments with Tumours

It was shown by Potter, Taylor and MacDowell (1938) that C58 strain mice could be immunized against a transplantable leukaemia by repeated inoculation of cells starting with sublethal doses, and that spleen and liver from mice immunized in this way could confer immunity* on others of the same strain. Subsequently MacDowell, Potter, Richter, Victor, Bovarnick, Taylor, Ward, Laanes and Wintersteiner (1938) reported that the power of these tissues to confer immunity was greatly increased by hyperimmunization. Barnes, Hoecker and Case (1952) obtained somewhat similar results with a leukosis in another strain. They found that immunity could be transferred by spleen and thymus, and to a slight extent by lymph nodes.

Mitchison (1954) transplanted a lymphosarcoma which grew in CBA and C3H mice to two resistant strains, A and C57BL, and determined the period of survival by excising the transplants at intervals and testing their viability by replacing them in CBA mice. The median survival time was 9.9 days in non-immunized adult mice and 2.2 days in mice which had been immunized by a previous transplant. Mitchison showed that immunity could be transferred from one mouse to another of the same strain by intraperitoneal injection of cells from the lymph nodes draining the area in which the tumour transplant was located.* To do this he investigated the viability of test transplants of the tumour after eight days in the lymph node recipients. By this test the power of the regional nodes to confer immunity persisted for less than 20 days after the original tumour transplantation, and the transferred immunity disappeared within 20 days of the injection of lymph node cells. Cells from the contralateral lymph nodes did not confer immunity.

In later experiments Mitchison (1955a) used the A strain sarcoma I and various tumours transplantable in other strains, and found that the regional lymph nodes acquired the power to transfer immunity within 0–5 days of the primary transplantation and lost it within 10–20 days, the precise times depending on the tumour and the strains of mice employed.

Only nodes which were markedly hypertrophied were able to confer immunity, and the minimal amount of lymphoid tissue mince required to transfer immunity to an animal of the tumour host strain was that harvested from three immunized animals. With cell suspensions the minimal effective dose was 50 million cells.†

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*Shown by the capacity to survive injection of a dose of tumour cells which would normally be lethal.

*These will be referred to as the regional lymph nodes.

†This figure corresponds to the regional lymph nodes from two [illegible] immunized animals. It was arrived at by using a [illegible] with test of immunity which was slightly [illegible] than the regular test used in other of Mitchison's experiments.

The duration of the transferred immunity was limited to between 3 and 15 days when the lymph node donor and recipient were from different strains (differing at one or more histocompatibility loci but not at the H-2 locus*) but was distinctly longer when both animals were members of the same strain.

Freezing and thawing of the transferred cells prevented transfer of immunity. Cells from other nodes and from the spleen, and whole blood or serum failed to transfer immunity. Cells from the regional nodes of animals bearing second grafts were tested on the 2nd, 3rd, 4th, 5th, 7th and 10th days. They appeared to be much less effective than cells from the regional nodes of animals bearing first grafts, but some effect was observed with all except those taken on the 10th day.

At first sight the simplest explanation of the transfer of immunity to tumour transplants by lymph node cells would seem to be that tumour cells pass from the tumour to the lymph nodes of the primary tumour host, are transferred with the lymph node [illegible] induce active immunity in the [illegible] hosts. Mitchison has adduced [illegible] reasons however why this must be [illegible]. In the first place the lymph nodes [illegible] transplanted into susceptible mice never gave rise to tumours. Secondly, active immunity induced by tumour cells took longer to develop than that resulting from transfer of lymph node cells. Thirdly, lymph node cells from an immunized animal differing genetically from the tumour donor only in respect of a single histocompatibility (H-2) gene failed on transfer to increase the natural resistance to the tumour of a third animal having the same genetic constitution as the tumour donor in respect of the H-2 locus but differing in respect of other histocompatibility genes. This would seem to be inconsistent with the transfer of antigen hypothesis unless we postulate further that the antigens are sorted out in the primary tumour host so that only those capable of immunizing this animal enter, and are subsequently transplanted with, the cells of the regional lymph nodes. Finally, lymph node cells conferred protection against tumour homografts on secondary hosts whose resistance to the tumour in question had been blocked by pretreatment with frozen or lyophilized tissue (p. 131).

Mitchison rejects also the hypothesis that the transferred immunity is conferred by preformed antibody carried by the cells and released into the serum of the host, on the ground that if such antibody is effective in combating grafts of tumour it should have been detectable in the blood of the primary hosts, and therefore whole blood or serum might be expected to confer immunity.

There remain two possibilities: (a) that the lymph node cells are immunologically activated before they are transferred, and confer immunity by continuing to function in the secondary host; and (b) that material in the form of either transformed antigen or an enzyme system for the manufacture of antibody passes from the transplanted lymph node cells into the cells of the host and activates them immunologically. There is at present no decisive evidence in favour of one as against the other of these two hypotheses, and it is noteworthy that similar uncertainty obtains with various other systems of transfer of immunity by cells (*see* Fagraeus and Grabar, 1953; Chase, 1953; Mitchison, 1955a). The observation of Jeter, Tremayne and Seebohm (1954) that delayed hypersensitivity to 2,4 dinitrochlorbenzene can be transferred by material obtained from disintegration of cells by sonic vibration, and the claim of Sterzl and Hrubesova (1956) to have transferred the

*As Mitchison (1955a) pointed out difficulties beset any attempt to measure the duration of immunity transferred into a foreign strain because the transferred cells may incidentally evoke a state of active immunity against the tumour if the tumour recipient and the tumour have antigens in common. This determined the choice of strains in his experiment.

power to form antibodies by means of nucleoprotein fractions suggest however that the second hypothesis certainly cannot be disregarded

Experiments with Normal Tissues

Billingham Brent and Medawar (1954b) immunized CBA mice with homografts of skin or other tissues from A line donors and then investigated the capacity of lymph nodes spleen leucocytes whole blood and serum from these primary hosts to confer immunity on other CBA mice In all experiments making use of the transfer of lymph node or splenic tissue each primary host was immunized by a single skin homograft transplanted to a defect on the right hand side of the chest (a region from which the lymphatic drainage is to the neighbouring axilla) The primary hosts which were donors of leucocytes whole blood or serum were immunized by either one or two skin homografts or by massive intraperitoneal inoculations of small fragments of donor spleen kidney lymph node or other tissue

It was found that heightened resistance to skin homografts was conferred upon the secondary hosts by the intraperitoneal inoculation of chopped fragments or expressed cells derived from the regional lymph nodes of actively immunized primary hosts but subcutaneous inoculation was completely ineffective The power to confer heightened resistance was abolished by freezing and thawing freeze drying or extracting the nodes by grinding with sand under normal saline

A dosage of only two nodes was ineffective and under similar conditions of administration eight nodes were decidedly superior to four Dose for dose more strongly positive results were obtained by transferring the lymph nodes three days prior to test skin grafting than by carrying out these procedures simultaneously Nodes removed 11 days after primary grafting were used in most of the experiments but it was also shown that heightened resistance was conferred by nodes removed after 30 days The secondary hosts still showed heightened resistance 15 and 30 days after lymph node transfer but on the other hand even an eight node inoculum did not modify the behaviour of a test graft which had already been in place two days before the nodes were transferred

Splenic tissue was effective in relatively high dosage but the non regional lymph nodes (viz the contralateral axillaries the cervicals and the homolateral inguinals) were at most feebly active Blood leucocytes whole blood and serum were completely ineffective

Billingham *et al* discuss the same four conceivable explanations of the phenomenon as Mitchison (*v supra*) and reach the same conclusions except in so far as they have a decided preference for the hypothesis that the transferred cells survive and continue to function immunologically in the secondary host and are inclined to regard the hypothesis of activation of host cells as an unnecessary complication

One of the reasons Mitchison advanced for rejecting the hypothesis of antigen transfer was that lymph node cells conferred resistance to tumour homografts on secondary hosts which had been pretreated with frozen or lyophilized tissue As we shall see (Chapter 6) this pretreatment only slightly reduces an animal s resistance to homografts of normal tissue but complete tolerance to such homografts can be induced in another way Billingham *et al* took advantage of this fact to perform an experiment similar in principle to Mitchison s but more decisive

THE SEARCH FOR HUMORAL ANTIBODIES

The search for antibodies in the sera of homotransplant recipients has yielded astonishingly different results in different hands. Until recently many investigators *doubted the existence of such antibodies*, but the patient investigations of P. A. Gorer (for review see Gorer, 1956) and others have done much to overcome this scepticism. There is still, however, wide difference of opinion concerning the conditions under which such antibodies can be found, and their role—if any—in the homotransplant rejection mechanism.

The tests which have been used may be classified as follows:

In Vitro Tests
- Tests for haemagglutinins and haemolysins
- Tests for cytotoxic antibodies
- Complement fixation and precipitin tests

Tests Performed Partly or Wholly In Vivo
- [illegible] immunization tests
- [illegible] test
- [illegible] Forman test

[illegible] *of investigating the role* of [illegible] in homograft destruction [illegible] the fate of grafts enclosed in [illegible] walls are permeable to [illegible] but not to cells. Experiments [illegible] kind will be considered in the next chapter.

Terms like *agglutinin, haemolysin, cytotoxic antibody, complement fixing antibody* and *precipitin* are commonly used and are convenient, but it must be emphasized that the use of a variety of terms does not necessarily mean that we are dealing with an equally diverse range of antibodies. It would take us too far afield to consider at length the vexed question of the unity and diversity of antibodies, and the reader who is interested is referred to an excellent review by Kabat (1953).

Two other terms which call for comment are *iso-antibody* and *iso-antigen*. They are commonly used to denote antibodies formed in consequence of transplantation of homologous cells (including erythrocytes), and the antigens which evoke them. Clearly it would be more appropriate to speak of *homo-antibodies* and *homo-antigens*, but to attempt to change the terminology would probably only add to the confusion.

Tests for Haemagglutinins and Haemolysins

Observations with Tumour Transplants

It was discovered by Lumsden (1937, 1938) and Gorer (1937, 1938) that red cell agglutinins appeared transiently in the host's blood during or immediately after the regression of a tumour transplant. Subsequently Gorer (1942) found that, following injection of homologous leukaemic cells to mice naturally resistant to them, two distinct antibodies appeared in the recipients' serum: one a haemagglutinin, the other a neutralizing antibody (p. 90).

More detailed studies (Gorer, 1947, 1950) using a whole battery of tests, including blocking tests (Wiener, 1944; Race, 1944), the conglutinin test of Wiener (1945), the albumin test of Diamond and Denton (1945), and the Coombs test (Coombs, Mourant and Race, 1945), led Gorer to conclude that there are at least four types of antibody formed in mice following tumour homotransplantation: (a) ordinary agglutinins which react in saline; (b) antibodies that require a high concentration of mouse serum to cause agglutination; (c) agglutinins inactive in mouse serum but active in human serum; (d) antibodies giving a prozone (Boyd, 1956; Race and Sanger, 1950) in heated tumour serum, but lysis with heated human serum. Types (c) and (d) are known as *incomplete* or *blocking antibodies* (Wiener, 1944; Race, 1944). They

were first included by Gorer in a single category and were recognized by their capacity to inhibit the action of ordinary agglutinins

It might be thought that these findings could be attributed to the antigenic effect of erythrocytes included accidentally with the tumour transplants but this is disproved by the following ingenious experiment of Kaliss and Jay (1950) These investigators transplanted a tumour indigenous to strain A mice to C57 black mice and showed that haemagglutinins developed in the hosts sera They next transplanted the tumour to unrelated inbred strains in which it took in a small proportion of cases the tumours thus formed which now contained foreign erythrocytes were then transplanted to C57 blacks Sera from these blacks were later found to agglutinate strain A erythrocytes whereas normal C57 serum agglutinated neither It was concluded therefore that the agglutinin formed in C57 recipients must have been evoked by antigens in the tumour tissue

In the course of investigating the agglutination of mouse cells in the presence of human serum Gorer found that suitably absorbed sera from patients with myelomatosis gave particularly satisfactory results and when it was found that one of the abnormal protein constituents in this serum contained a high proportion of carbohydrate Gorer and Mikulska turned to a study of various naturally occurring mucoproteins and mucopolysaccharides to determine whether they could be used in conjunction with absorbed normal human serum Pseudomucin from ovarian cysts gave the best results but the lack of a regular supply of this material led to the search for a substitute and Gorer and Mikulska (1954) found this in dextran having a molecular weight of 100 000 or less *

Gorer and his colleagues (see Gorer 19[illegible] have since used the dextran test extensively to investigate the formation of red cell agglutinins following transplantation of tumours to mice differing at the H 2 locus from the donor strain They have found with a variety of tumours that haemagglutinins may appear on the third day after transplantation are easily detected on the fourth day reach a maximum titre about the time that the transplant is beginning to regress and may persist for weeks or months These observations are at variance with those of Mitchison and Dube (1955) who were unable to detect haemagglutinins until regression was complete but Gorer (1956) has suggested that the discrepancy may be due to the fact that the dextran used by these authors gives a very insensitive system They are also in sharp contrast with the observation of Schneweis and Knake (1958) that in rats there was no correlation between the behaviour of a tumour transplant and the presence or absence of isoagglutinins

The significance of red cell isoagglutinins in the sera of recipients of tumour homotransplants is thus difficult to assess It seems clear that in mice the genes of the H 2 locus determine not only histocompatibility but also iso antigenic differences (p 81) which can be detected by red cell agglutination tests Gorer at one time inclined to the view that the agglutinins evoked by these antigens were responsible for the destruction of tumour homotransplants It is now clear that this is not true of homotransplants of tumours in general for they may grow progressively despite a high antibody titre (Gorer 1947 1948) and under certain conditions their growth may be enhanced by injection of immune serum (p 132) it may however hold good to a large extent for the leukoses

Antibodies may also be detected in the [illegible] of mice which have received tumour [illegible] transplants by testing for red cell hae[illegible] in the presence of complement and

* [illegible]

by leucocyte agglutination (Amos, 1953), but these tests have not been widely used. Since normal mouse serum usually shows little or no complementary activity and isoimmune sera may be strongly anticomplementary, care must be taken in testing for [illegible] activity to see that an adequate amount of complement is added to each tube [illegible] the test may be made more sensitive by adding also saline containing 0.01 [illegible] of Mg++ (Hildemann, 1957b). [illegible] unknown reason mouse sera tend [illegible] on storage a non-specific in[illegible] which interferes with leucocyte agglutination, but fortunately this can be [illegible] by emulsions of liver and other [illegible] the strain supplying the serum.

Observations with Transplants of Normal Tissues

[illegible] 1946b) was unable to detect [illegible] in rabbits which had [illegible] skin homografts On the [illegible] Gorer, Mikulska, Billing[illegible] (1951) found that full [illegible] homografts in mice, and also [illegible] epidermis, stimulated the [illegible] antibodies capable of agglu[illegible] cells and leucocytes from [illegible] Absorption tests suggested [illegible] antigens were present [illegible] leucocytes, but that some [illegible] absent from the red cells [illegible] 1955).

[illegible] 53a) found that incomplete antibodies against the donor's erythrocytes could sometimes be demonstrated in dogs which had received a kidney homotransplant by vascular anastomosis, although he did not think that they played any part in the destruction of the transplant.

Clarkson and Gorer (1956) found isohaemagglutinins in the serum of a seven year old child who had received skin homografts from her father following a severe burn. The blood of both the donor and the recipient was group A, Rh+. The homografts sloughed after 14-20 days and were replaced by autografts. The child was substantially healed 60 days after grafting and about this time antibody reacting with the father's erythrocytes was detected in the child's serum, first by the indirect Coombes test, and subsequently by agglutination in compatible human serum diluted with 10 per cent dextran (Intradex, salt free). The titre was low but antibody persisted for at least three months.

In Vitro Tests for Cytotoxic Antibodies

One procedure which has been widely used is to culture* small explants of donor tissue *in a medium containing the host* serum to be tested, and compare their behaviour with that of similar explants grown in a medium containing instead serum from an animal of the same species which has not received a transplant. The comparison may be based on morphological and physiological characteristics such as the rate of cell migration and the frequency of cell division (Lambert and Hanes, 1911c; Medawar, 1948c), and the metabolic activity of the explants; or, alternatively, on the ability of the explants to survive when transplanted back to the original donor.

Another method is to study the effect of adding the serum to be tested to a suspension of viable donor cells There is evidence that when cells die they become permeable to various dyes such as trypan blue, eosin and safranin (Pappenheimer, 1917; Richter and MacDowell, 1933; Schrek, 1943a, b, 1944a, b, 1945, 1946a, b, 1948, 1949, 1952, 1953), and if this is true it follows that the number of living cells per c.mm. in a suspension may be determined by adding an appropriate dye and then counting the unstained cells with a haemocytometer. In performing the test equal quantities of the

*Tissue cultures are commonly grown in media consisting of water, electrolytes, homologous or heterologous plasma, and homologous or heterologous embryo extract (*see* Parker, 1950).

cell suspension are placed in a number of tubes. The serum to be tested is added to some of the tubes and an equal quantity of control serum to the remainder. Complement* is added to some or all of the tubes in both groups. After thorough mixing samples are withdrawn from each tube and diluted with a solution of dye in a leucocyte pipette, and the unstained cells are counted in a haemocytometer. The tubes are then incubated at 37°C, and further unstained cell counts are made after 6, 12 and 24 hours.

Observations with Tumour Transplants

Lumsden (1937, 1938) found that when the serum of animals bearing tumour transplants contained demonstrable haemagglutinins (p. 84) it also had a cytotoxic effect on donor macrophages and tumour cells in tissue culture. This conflicts however with the earlier work of Lambert and Hanes (1911a), and also with the later investigations of Harris (1943b), who reported that the sera of rats and mice which had received tumour homotransplants had no effect on the corresponding tumour cells in tissue culture, and Kidd (1946), who found that serum from rabbits which had been immunized with tumour homotransplants and gave a positive complement fixation test, had no visible effect on the tumour cells in suspension, nor did it appear lethal to these cells as judged by the method of unstained cell counts, whether complement was present or not. Moreover it has been found that the Brown-Pearce carcinoma may be cultured as readily in the serum of a rabbit immune to this tumour as in normal serum (Favorite and Cheever, 1941), and oxygen consumption, respiratory quotient, anaerobic and aerobic glycolysis, glucose utilization and lactic acid production are the same in both cases.

Still more recently, however, Kalfayan and Kidd (1953) have reported that serum from rabbits immunized against the Brown-Pearce carcinoma, when mixed with complement, causes gross structural changes in the tumour cells *in vitro*, and Gorer and O'Gorman (1956) have shown by the unstained cell count test that the H-2 antibodies in mice have a cytotoxic effect on donor strain spleen cells and tumour cells, so that the pendulum appears to be swinging in the opposite direction again.

Observations with Transplants of Normal Tissues

Medawar (1948c) tested the sera of rabbits which had received homografts of normal skin by incubating each serum with a suspension of epidermal cells from the corresponding donor for 3-6 hours at 37°C and then allowing the mixture to stand overnight at room temperature. No agglutination or other evidence of cellular damage was observed. This negative finding was later confirmed by Allgower, Blocker and Engley (1952).

Tests performed with tissue cultures have also yielded negative results. Harris (1948) found that explants of rat heart grew normally in a medium containing serum from rats which had received a homotransplant of normal tissue, and Medawar (1948c) found similarly that rabbit skin could be cultured in a medium containing serum from rabbits heavily and specifically* immunized against it, and showed the same degree of migratory and mitotic activity in this medium as in a medium containing normal instead of immune serum. Medawar showed further that explants of donor skin behaved similarly when host leucocytes, defibrinated blood or tissue extracts were

* As we have seen complement must be present before haemolysins can bring about lysis of red cells. Much less is known of the role of complement in other cytolytic systems but negative results should not be regarded as significant unless complement is added in an amount sufficient to overcome any anticomplementary activity of the serum being tested (Gorer and O'Gorman 1956).

* In this experiment the explant and the corresponding immunizing graft were in every instance *obtained* from the same donor.

added to the culture medium, or when they were cultured in close contact with host reticulo-endothelial tissue in a medium containing host serum. This last finding seems all the more remarkable in the light of the observation already described that the survival of homotransplants of normal thyroid in the anterior chamber of the eye is prevented if a piece of autologous splenic tissue is placed in juxtaposition with the homotransplant.

Another test based on the study of living cells *in vitro* was used by Sparrow (1954), who incubated donor leucocytes in serum from an immunized host to which were added also lymphocytes from the draining lymph nodes of the same host. It was found that the phagocytic activity of the donor leucocytes was unaffected by this treatment.

More recently still Hulliger and Allgöwer (1957) have shown that cultures of donor buffy coat leucocytes in the plasma of rabbits immunized by either a large skin homograft or repeated intradermal injection of [illegible] leucocyte suspensions, are indistinguishable [illegible] in appearance—and in particular in their [illegible] capacity to form a network of fibroblasts [illegible] from cultures in the donor's own plasma [illegible].

Complement Fixation and Precipitin Tests

[illegible] fixation tests are performed in the usual way (*see* Boyd, 1956) but, as Maltaner (1950) and others have pointed out special precautions may be required if the sera under investigation show marked anticomplementary activity.

Precipitin tests may be performed in the classical way or alternatively the method of immuno electrophoresis devised by Grabar and Williams (1955) may be used.

Observations with Tumour Transplants

Kidd and his colleagues (Friedewald and Kidd, 1945, McKenzie and Kidd, 1945; Kidd, 1946) reported that the sera of rabbits bearing transplants of certain tumours contained complement-fixing antibodies which reacted *in vitro* with a distinctive sedimentable constituent of the corresponding tumour cells. The antibodies evoked by two of the tumours studied (designated the V_2 carcinoma and sarcoma I) reacted not only with constituents of the corresponding tumour cells but also with constituents of another tumour and of various normal rabbit tissues, whereas the antibody evoked by the Brown-Pearce carcinoma, on the other hand, appeared to be specific for this tumour.

Kidd (1946) reported further that antibody sometimes appeared in the serum of animals which had received injections of cell-free tumour extracts. Animals so treated which developed the antibody were resistant to subsequent tumour homotransplants in the form of intramuscular injections of suspensions of viable tumour cells; animals which failed to develop the antibody, on the other hand, were not resistant to transplants.

Maltaner (1950) has suggested that the positive complement fixation tests obtained by Kidd are invalid, being due to "the activation of the accelerator globulin in the rabbit sera by the . . . tissue extracts used as antigens." Further work would appear to be required to determine whether this criticism is justified.

Observations with Transplants of Normal Tissues

Medawar (1948c), and Allgöwer, Blocker and Engley (1952), tested the sera of rabbits which had received skin homografts for precipitins by mixing each serum with an aqueous extract of donor skin, but the results were completely negative.

Simonsen (1953a) too failed to demonstrate complement-fixing antibodies* in

*Simonsen found, as Kidd and Friedewald (1942) had found in rabbits, that fixation of complement occurred when normal dog serum which had been heated to 56°C. was mixed with an aqueous extract of either autologous or homologous kidney. There was, however, no significant change in the strength of this reaction after transplantation.

host serum against renal tissue from the donor after transplantation of whole kidneys by vascular anastomosis although, as we have seen (p. 86), incomplete haemagglutinins were sometimes present.

In contrast to these negative results it has been shown in rabbits that antibodies which react with homologous tissue or tissue extracts may be formed if homogenates of *homologous* tissue are injected together with staphylococcal or streptococcal toxin. Thus Schwentker and Comploier (1939) injected rabbits with a mixture of homologous kidney emulsion and staphylococcal or streptococcal toxin, and found that complement fixing antibodies were formed which would react with rabbit kidney and brain. Similarly Hecht, Sulzberger and Weil (1943) injected rabbits with minced homologous skin together with staphylococcal toxin, and found that a precipitin was formed which would react with a soluble antigen obtained from autolysed rabbit skin. It is noteworthy that in neither of these investigations was the homologous tissue by itself effective. The significance of the observations is not altogether clear because no attempt was made to immunize the host against *one* particular donor and then perform tests with host serum and donor antigen. It may well be, therefore, that the antibodies formed were tissue-specific rather than individual-specific.

More recently, however, Bollag (1956) claims to have demonstrated individual-specific precipitins by a zonal turbidity reaction with extracts of donor tissue in the sera of rabbits immunized by skin homografts without the aid of adjuvants. The antibody appeared on average 7 days after grafting and disappeared after about 15 days.*

*It is not clear from Bollag's paper whether the antibodies disappeared 15 days after grafting or 15 days after they first appeared.

Passive Immunization Tests

Two tests are available.

The first consists in injecting the serum under investigation into a normal animal of the same species, and later grafting from the original donor to this animal to determine whether immunity has been passively transferred.

The second test is an adaptation of the *Prausnitz-Kustner reaction* (Prausnitz and Kustner, 1921; *see also* Boyd, 1956), which was used originally to demonstrate antibodies (then termed *reagins*) in the sera of patients who showed allergy to pollens and other substances. The test is performed by injecting a small quantity of serum (usually 0.1 ml.) intradermally to a normal individual of the same or another species, and 24-48 hours later injecting a small quantity of the antigen at the same site. Appropriate control injections are used to eliminate the risk of obtaining false results due to the test animal (or human subject) being sensitive to either the serum or the antigen alone. A positive result, shown by the development of an inflammatory reaction at the test site in the absence of any reaction at the control sites, indicates that the immunity has been passively transferred by the serum to the site of injection in the test animal.

Observations with Tumour Transplants

MacDowell and his colleagues (Richter and MacDowell, 1935; Potter, Taylor and MacDowell, 1937) tried unsuccessfully to transfer immunity to a transplantable leukaemia by means of serum. The same test was applied to lymphosarcoma transplants by Stoerk (1953b) and Mitchison (1953, 1954) Stoerk reported successful passive transfer by serum whereas Mitchison obtained negative results.

More recently successful transfer of immunity to leukoses by intraperitoneal injection of serum has been reported by Gorer and Amos (1956) and Amos and Day (1957). It appears from these experiments that anti-

bodies prepared by hyperimmunizing mice of a resistant strain against a transplantable leukaemia or lymphoma will passively immunize mice of both resistant and susceptible strains Absorption tests suggest that *two independent groups of antigens are concerned in the immunization process*, namely the antigens of the H-2 system, which are present in normal donor tissues as well as in the tumour, and antigens termed by Gorer and his colleagues *X-type antigens*, which are confined to the tumour and are distinct for each tumour so far investigated Immunity due to antibodies against the H 2 antigens can be transferred passively to resistant mice but not to susceptible mice, whereas antibodies against the X-antigens confer protection when transferred to any strain.

Observations with Transplants of Normal Tissues

Billingham, Brent and Medawar (1954b), as we have seen (p. 83), were unable to transfer immunity to skin homografts in mice passively with serum, and Dempster (1953a) found that serum from dogs which had received kidney homotransplants had no nephrotoxic or other demonstrable effect when injected to the graft donor

The Neutralization Test

The neutralization test consists in incubating donor tissue or cells in the serum under investigation and subsequently transplanting the material homologously or isologously to a normal animal The behaviour of these transplants is compared with that of control transplants pre-incubated in normal serum.

Observations with Tumour Transplants

Gussio (1911) and Mottram and Russ (1917), applied the neutralization test to the serum of tumour bearing rats, and Lench (1920) performed similar experiments in mice, but the results were entirely negative.

The first positive results were obtained by Gorer (1942), who found that, following injection of homologous leukaemic cells to mice naturally resistant to them, two distinct antibodies appeared in the recipients' serum. One was a haemagglutinin; the other, which was termed by Gorer a protective antibody and could be separated from the agglutinin by absorption, had no visible effect on the leukaemic cells in suspension but rendered them more or less innocuous as judged by their behaviour when subsequently transplanted to a suitable host.

Further positive results have been reported with mouse leukaemia (Mitchison, 1955b), and with various other tumours including fowl leukaemia (Burmester, 1947), the Brown-Pearce carcinoma of rabbits (Kidd, 1946), and a mouse sarcoma (Mitchison and Dube, 1955).*

On the other hand Kidd and Toolan (1950) found that the ability of cells of a transplantable mouse lymphosarcoma to induce tumours when injected to mice of a susceptible strain was not impaired by prior incubation in serum from non-susceptible mice immunized with this tumour. They did find, however, that the cells failed to produce tumours after being mixed with a suspension of minced lymphoid tissue from immunized mice and incubated for two hours. Similar results have been obtained by Stoerk (1953b) in rats. The serum of animals immunized with a transplantable lymphosarcoma failed to damage lymphosarcoma cells in suspension, as judged by their ability to produce tumours when transplanted to a susceptible host; but these cells failed to produce tumours when mixed with viable lymphoid cells from immunized rats.

Observations with Transplants of Normal Tissues

Billingham and Sparrow (1954) incubated dissociated rabbit epidermal cells in the

*Mitchison and Dube found however that a haemagglutinin test gave more consistent results with the same tumour (the A strain Sa 1).

serum of rabbits which had received a skin homograft from the animal from which the cells were obtained. They found that the cells were "altered in such a way as to make them incapable or nearly incapable of reconstitution and growth when grafted back to a raw area in the donor."

The Woodruff-Forman Test

Woodruff and Forman (1950) transplanted lymphoid tissue and skin from hooded rats to Wistars, and three weeks later obtained serum from each recipient and injected 2.5 c.c. intraperitoneally into the corresponding donor. Total and differential leucocyte counts were made before and at intervals after the injection. Other Wistar rats served as controls and each received 2.5 c.c. of serum from a normal hooded rat. It was found that the absolute lymphocyte count fell in both the experimental and the control animals but that the fall was significantly greater in the former group. This effect was attributed to the presence of anti-lymphocytic antibodies evoked by the transplants, and it was concluded that there exists an antigen common to lymphoid tissue and skin. No explanation was suggested to account for the lesser but quite definite lymphocytopenia which occurred in the control animals, but it was realized later that this was almost certainly a stress phenomenon mediated by an adrenocortical mechanism. The whole experiment was therefore repeated in adrenalectomized animals (Woodruff, unpublished experiment) and it was then found that the leucocyte count remained unchanged following injection of either immune or control serum. It would thus appear that the original conclusion that serum from the homotransplant recipients contained anti-lymphocytic antibody was unjustified; the only conclusion warranted by the findings is that the serum of homotransplant recipients is modified in some unknown manner which renders it more stress-producing than normal serum when injected into the donor of the transplant. It seems likely, however, that this change in the serum is due to the presence of an antibody of some kind.

General Conclusions

There is no doubt that homotransplants of both neoplastic and normal tissues may stimulate the formation of humoral antibodies. These antibodies may be difficult to demonstrate however and the titre may not reflect the state of the transplant.

One explanation which has been suggested to account for this elusiveness is that antibodies formed in response to homotransplants are for the most part rapidly removed from the circulation and fixed in the tissue of, or in the immediate vicinity of, the transplant (Allgöwer, Blocker and Engley, 1952; Pressman, 1955). To test this hypothesis Allgöwer *et al.* added extracts of skin homografts, and of the graft beds, to tissue cultures of donor skin. The cultures were unaffected, but this does not necessarily mean that the hypothesis is untrue because in the first place the process of extraction may well have failed to remove cell-bound antibody from the tissue, and secondly, as we have seen, some forms of antibody are not detected by the tissue culture test.

Humoral antibodies may well be of special importance in the destruction of leukotic transplants, but they are not sufficient to accomplish the destruction of homotransplants of other types of tumour and of normal tissues, and it is not even certain that they are necessary.

The antigens which evoke the formation of iso-antibodies are not necessarily the antigens which determine the rejection of a homotransplant and the second-set phenomenon, but this is a matter we shall return to later (p. 93).

MANIFESTATIONS OF ACQUIRED SENSITIVITY

Introduction

Hypersensitivity reactions are of two main kinds the *immediate* or *early type*, and the *delayed type*

Immediate-type sensitivity may be manifested by a wheal and flare reaction or by the Arthus phenomenon when the agent responsible is injected into a sensitized recipient, and characteristically (though not invariably) the reaction reaches its maximal intensity—as judged by the macroscopic changes—almost at once or within an hour or two.

If the injection is intravenous there may be no local reaction but the condition known as *anaphylactic shock* may develop, the symptoms of which differ somewhat in different species (*see* Boyd, 1956)

Humoral antibodies play an important part in immediate-type sensitivity. In particular it has been shown by various tagging tests that soluble substances which provoke an antibody response are distributed widely through the body (Favour, 1957), the titre of antibody in the serum parallels the degree of sensitivity and sensitivity can be transferred passively by serum alone.

Delayed type sensitivity is exemplified by sensitization to tuberculin and to various simple organic substances such as picryl chloride The reaction which occurs when the agent responsible is injected into the skin of a sensitized recipient typically does not reach its maximal intensity (again judging by the macroscopic appearance) for 24-48 hours

Antibody may not be detectable in the serum, and if it is found the titre does not run parallel to the development of sensitivity. Generally speaking the sensitivity cannot be transferred passively by serum, although successful transfer by this means was reported once many years ago by Zinsser and Mueller (1925), and more recently Cole and Favour (1955) have shown that delayed-type sensitivity to tuberculoprotein, and immediate-type sensitivity to tuberculopolysaccharide may be transferred by separate serum fractions, but that when the two fractions are combined the antibody to tuberculopolysaccharide inhibits the passive transfer of delayed-type hypersensitivity.

Both types of hypersensitivity can be transferred passively by cells (Chase, 1945, 1950, 1953). Generally speaking, as we shall see in the next chapter, immediate-type sensitivity reactions are inhibited by antihistamine drugs but not by cortisone and allied substances, whereas delayed-type reactions are inhibited by cortisone but not by antihistamines.

Good, Varco, Aust and Zak (1957) were unable to demonstrate delayed-type sensitivity in patients with agammaglobulinaemia, but Kulneff, Pedersen and Waldenström (1955), and Porter (1957), on the other hand, observed the development of tuberculin sensitivity in agammaglobulinaemic patients inoculated with BCG, and Porter also induced delayed-type sensitivity with dinitrofluorobenzene in his patient.

The terms immediate-type and delayed-type sensitivity are so well established as to be almost unchallengable, but they must not be interpreted too literally because the macroscopic appearance of a sensitivity reaction may be misleading, and in so far as there is a fundamental distinction between the two types of sensitivity it rests on differences in the histological features of the local reaction and on the part played by humoral antibodies in the process.

Hypersensitivity and the Homograft Reaction

Barker (1918) tried to sensitize guinea pigs to extracts of homologous skin by giving three subcutaneous injections of the extract on alternate days. Two weeks later he injected 1 ml. of extract intravenously but

this did *not* cause anaphylactic shock. On *the other hand* Allen, Williams, Lovingood and Ellison (1952) found that rabbits rendered less resistant to homotransplants by prior injections of suspensions of killed donor skin (Chapter 7) developed fatal anaphylactic shock when subsequently injected intravenously with another dose of the same suspension.

A more direct approach to the problem is to perform skin sensitivity tests by making intradermal injections of donor skin extracts *to homotransplant recipients*. The author (*unpublished*) tried this some years ago in rats, but failed to obtain any evidence of skin sensitivity. This failure was probably due to an unfortunate choice of animal, and Brent, Brown and Medawar (1958) have now shown that delayed type hypersensitivity reactions occur when guinea pigs which have been immunized with homografts are injected intradermally with living cells (preferably lymphoid cells from the graft donor), and also with cell free antigenic material prepared from donor spleen cells by the method of Billingham, Brent and Medawar (1958).* They showed *moreover* that a reaction (termed a transfer reaction) occurs also when cells from the regional lymph nodes of a homograft recipient guinea pig are injected intradermally into the graft donor, but that host serum alone does not evoke a *reaction in the donor*. Both the direct and *transfer reactions* had a *latent period* of 5-8 *hours* and were maximal after 24-48 hours. Similar findings were obtained in rabbits, but experiments in mice gave negative results.

Another phenomenon of interest in the present context was reported by Rapaport and Converse (1957). They made repeated skin homografts to a human volunteer and observed that the rejection of each successive graft was accompanied by a *recall flare* at the site of the preceding graft.

*This is described on p. 91.

THE ANTIGENS CONCERNED IN HOMOGRAFT IMMUNITY

The acquired immunity hypothesis implies that there are antigens which determine the homograft reaction. Such antigens will be referred to as *transplantation antigens* or *T antigens*.

Experiments with successive transplants of different tissues from the same donor, as we have seen, suggest that different tissues of the same individual have T antigens in common. Experiments on the induction of specific immunological tolerance to homotransplants, which will be considered in Chapter 7, point to the more far reaching conclusion that all the normal nucleated cells in an individual possess the same set of T antigens.* Barrett, Hansen and Spilman (1951) claim that in mice these antigens are present also in erythrocytes, but others have not been able to confirm this.

It seems certain that T antigens from a *solid homograft travel to the regional* lymph *nodes, and it is commonly assumed* that they are conveyed there either in solution or suspension. It is difficult to *reconcile* this assumption with the fact that a homograft enclosed in a diffusion chamber (p. 111) whose walls are impermeable to cells, and the mammalian foetus *in utero*, which is isolated by the placental barrier (p. 188), do not ordinarily evoke homograft immunity in the host or mother, and the author therefore believes that the T-antigens are normally *transferred in* cells. They could

*Tumour cells have antigens in common with normal cells but they do not necessarily possess all the T antigens of the normal donor strain tissues (see Gorer 1948) and as we have seen (p. 40) they may possibly possess special antigens of their own.

conceivably be carried to the lymph nodes by migration of cells from the graft, but it seems more likely that they are carried by host lymphocytes.

The T-antigens are present in the nuclei of cells Billingham, Brent and Medawar (1956b) succeeded in obtaining antigenically active material in a cell-free aqueous solution by ultrasonic disintegration followed by centrifugation at 25,000 g., and in view of their solubility properties and the fact that they appeared in some experiments to be destroyed by desoxyribonuclease but not by ribonuclease, suggested that the antigens were in fact desoxyribonucleoproteins. Further investigations (Medawar, 1957; Billingham, Brent and Medawar, 1958) have shown that this conclusion is erroneous and that "whatever may be the natural state of the transplantation antigens DNA is not a necessary ingredient" On the contrary, the evidence suggests that the determinant groups of the transplantation antigens are related to the human blood-group substances, i e. are amino-acid polysaccharide complexes (Billingham *et al.*, 1958). The abolition of antigenicity by exposure to desoxyribonuclease in the early experiments apparently occurred because the treatment altered the vehicle on which the antigens were carried

In the more recent experiments antigenically active material was obtained in the following way. Cells were expressed from spleen, lymph node or thymus, and washed successively in 0 15 M NaCl, 0.05 M NaCl and water. The resulting gelatinous sediment was cut up with scissors, resuspended in distilled water, and then rendered into a smooth viscous "solution" in a blender of the piston and cylinder type. The DNA-protein responsible for the viscosity of the material was then degraded and dispersed by exposure to ultrasonic vibration and after centrifugation at 2500 g. for 10 minutes to remove miscellaneous cellular debris the concentration of NaCl was restored to 0.15 M. The precipitate of DNA-protein which formed was removed by again centrifuging at 2500 g. for 10 minutes. The supernatant fluid, now free from cells or large particles, was then spun at 25,000 g. for 60 minutes, and the supernatant fluid discarded. The sediment, which was antigenically active, was re-suspended in Ringer-phosphate.

The antigenic power of this material was found to be reduced by freeze-drying and by heating to 56°C. for half an hour. It was abolished by precipitation with ethanol from a solution in acetate buffer, by incubation at 37°C. for an hour with an extract of *Trichomonas foetus* which is known to contain an enzyme which inactivates the human blood-group substances, and by exposure for two hours at room temperature to buffered 0 005 M potassium periodate—a procedure also known to destroy the human blood-group substances. On the other hand the material was stable to the action of ribonuclease, desoxyribonuclease, alpha amylase, beta amylase, hyaluronidase, and a highly purified preparation of receptor-destroying enzyme from *Vibrio cholerae*.

It is apparent from the work of Gorer and others (p. 81) that at least some homotransplants possess antigens which stimulate the formation of haemagglutinating antibodies, and these will be referred to as H-antigens. It seems clear moreover that the H-antigens are determined by the histocompatibility genes which determine the T-antigens, and it is natural to ask whether in fact the two sets of antigens are identical. As Medawar (1958, 1959) has pointed out however there are sound reasons for believing that they are distinct. In the first place the H-antigens appear to be chemically much more stable than the T-antigens; they are, for example, not destroyed by freezing and thawing tissues, by heating them to 50°C, or by freeze-drying them, whereas all these procedures greatly weaken or abolish the power of cells to produce transplantation immunity. Secondly, they are present

on the surface of red cells and in the cytoplasmic fractions of nucleated cells (i e fractions in which T antigens have not so far been identified) Thirdly, in mice at any rate, they appear later in development. Finally, Hildemann and Medawar (cited Medawar, 1959) have been unable to provoke the formation of haemagglutinins by injecting mice with nuclear fractions of cells which elicit transplantation *immunity*, or *to* absorb with these nuclear fractions haemagglutinins produced by the injection of living whole cells or lyophilized *tissues*

To *account* for this rather paradoxical state of affairs Billingham, Brent and Medawar (1956b) have suggested *that the* T- and H antigens stand to each other in the relationship of intermediate and secondary gene products.

It is worth mentioning in the present context that the assumption that the nuclei of differentiated somatic tissues are equal as to their genetic *factors* has not passed unchallenged (*see* Schechtman and Nishihara, 1955), and it may be that the generalization *that all the somatic nucleated* cells of *an individual* are antigenically *completely* identical will also have to be re examined

EFFECTOR MECHANISMS

Homograft immunity is a phenomenon *sui generis* Its closest affinity is with delayed type bacterial sensitivity (Lawrence, 1957), for both can be transferred by cells but not ordinarily by serum, both are inhibited by cortisone and related substances (Chapter 6), and in neither do serum antibodies appear to play a major role * There are, *however*, important differences, for homograft immunity, unlike delayed *type sensitivity, cannot* be transmitted by exudate cells *or blood* leucocytes but only by cells from the regional lymph nodes, or, in the case of hosts immunized by the intravenous route, from *the spleen* (Medawar, 1959) Moreover it looks as if the homograft reaction *is in abeyance in patients* with congenital agammaglobulinaemia whereas delayed type sensitivity reactions can occur, although there is some conflict of evidence about this

It has already been suggested (p. 94) that host cells may be concerned in the transport of antigenic material from homografts The evidence we have been considering, together with the observation *described* in the next chapter that homografts in diffusion chambers behave like autografts even in immunized hosts, suggests even *more strongly that* host cells are essential for the effector side of the homograft rejection mechanism

It seems likely that lymphocytes are of *special importance* One view of their role is that they are vectors of antibody, and some hypothesis of this kind seems inescapable, *but, as Medawar* (1958) has pointed out, it would be unwise to assume that they are "merely bags of serum antibody waiting to be set free " The *breakdown of homo* grafts is commonly associated with arrest of the circulation in the graft, and this *may be due to some kind* of reaction in *the* vas cular *endothelium* Histiocytes and fibro blasts also appear to play some part in homograft rejection, and it may well be that all these cells are sensitized by *contact with*, and possibly by transfer of material from, activated lymphocytes

One curious and as yet unexplained anomaly *is that second set skin* homografts, and as far as *is known free second set homo*grafts of other vascular tissues, show little evidence *of cellular infiltration and seem*

*Here, and in what follows, we are speaking of the reaction to homografts of normal tissues and solid tumour homografts. The reaction to leukotic grafts, as we have seen (p. 67), may be of a different kind

to perish as a result of ischaemia, whereas thin grafts in diffusion chambers, as we have already noted, may survive even in immunized animals.

Another apparent anomaly was reported by Mitchison and Dube (1955), who tagged activated lymph node cells from mice immunized by tumour homotransplants by staining them with acriflavine, and injected them intraperitoneally to animals of the host strain bearing subcutaneous transplants of the same tumour. Tagged cells could subsequently be seen in the spleen, liver, kidneys, lungs and mediastinal lymph nodes. They could not be found in the thymus, the axillary and inguinal nodes, or the transplants but despite this it was apparent from the behaviour of the transplants that immunity had been effectively transferred.

There seems little prospect of reaching a deeper understanding of the role of lymphocytes in homograft immunity, or in delayed type hypersensitivity reactions, until more is known of the normal function of these cells.

Hamilton (1956) has concluded from a study of the incorporation of adenine-8-carbon 14 in leukaemic lymphocytes that they either live much longer than is commonly supposed or else they re-utilize large fragments of nucleic acid or nucleoprotein from their progenitors. He has suggested that re-utilization of nuclear material may be characteristic of normal lymphocytes, and that if so it may be a means of preserving the necessary templates for the production of specific antibodies. This hypothesis, or some modification of it, may well prove fruitful in the field of homograft immunity.

The observation of Gowans (1957) that the fall in output of lymphocytes from a thoracic duct fistula in the rat can be prevented by continuous intravenous reinfusion of autologous lymph and living lymphocytes, but not dead lymphocytes, is not necessarily incompatible with the re-utilization hypothesis because, as Gowans himself suggests, the methods used for killing the cells (heat or exposure to ultraviolet irradiation) may have destroyed substances which were essential for new lymphocyte production, or alternatively the breakdown products of the dead lymphocytes may not have been deposited in the correct place for them to be effective.

While the emphasis at present is on the role of cells in homograft immunity it would be unwise to assume that except in the case of leukotic transplants serum antibodies play no part, and some of the author's experiments using diffusion chambers suggest that humoral antibodies may function as *opsonins* and facilitate the destruction of homografts by host cells (Woodruff, 1957b). The evidence for this, which is far from conclusive, will be considered in the next chapter.

GRAFT ADAPTATION

So far in discussing homograft immunity we have spoken as if the antigenic structure of the grafted cells is unchanging, and it is indeed commonly assumed to be unchangeable. There are however several observations which cast doubt on this dogma.

In the first place, as we have seen (p. 79), there is strong evidence that homografts in the anterior chamber of the eye (Woodruff and Woodruff, 1950), and probably also orthotopic corneal homografts (Maumenee, 1951), become less vulnerable as time goes on and after a certain critical period are capable of surviving in the face of a degree of immunity in the recipient which they would not have been able to withstand earlier in their life history (Woodruff, 1952a, 1954c).

Secondly, it sometimes happens that a second set graft, transplanted before a previous graft from the same donor has broken down, is destroyed while the original graft remains alive This was first reported by Longmire, Stone, Daniel and Goon (1947) in a human patient who received simultaneous skin homografts from many different donors After 6 weeks only one graft remained alive and a second graft from the donor of this surviving graft was transplanted to a previously ungrafted area The second graft took, but began to ulcerate after 9 days After 15 days it was completely destroyed and only then did the first graft show any signs of reaction The same phenomenon can be seen in an even more striking form in animals which have been made almost, but not quite, completely tolerant of donor tissues (p 128), for then a first graft may survive permanently while a second graft from the same donor is rapidly destroyed (Woodruff, 1957a)

Finally, as Cannon (1957) has reported, skin homografts to day old chicks—which survive permanently in 5 10 per cent of hosts—are sometimes rejected as late as 2 months after grafting, but never after 3 months Moreover not more than 50 per cent of grafts, when grown to adult age, can be returned to the donor without evoking a reaction

The nature of the adaptive process has not been elucidated It may depend on changes in the graft stroma, and on whether the graft is vascularized by invasion of host vessels or by the development of anastomoses between the vessels of graft and host At the present time, however, when the possibility of transfer of chromosomal material between cells is being seriously investigated, it would be unwise to deny without further investigation the possibility of a fundamental change in the antigenic structure of the grafted cells

CHAPTER 6

The Effect of Various Experimental Procedures on the Behaviour of Homotransplants

In this chapter we shall consider the effect of various experimental procedures on the behaviour of homotransplants, excluding induction of specific immunological tolerance which forms the subject of Chapter 7. They may conveniently be subdivided into three groups: procedures which affect primarily the host, procedures which affect primarily the transplant, and procedures designed to create a barrier between transplant and host.

PROCEDURES AFFECTING PRIMARILY THE HOST

The procedures to be considered are as follows:

1. Irradiation
2. Administration of chemical cytotoxins
3. Administration of cytotoxic sera
4. Reticulo-endothelial blockade
5. Splenectomy
6. Excision of the regional lymph nodes.
7. Administration of corticosteroids and ACTH
8. Administration of anti-histamine drugs
9. Administration of anticoagulants.
10. Administration of substances which lower the titre of properdin.
11. Thyroidectomy and hypophysectomy.
12. Alteration of the body temperature.

Total Body Irradiation

It has long been known that irradiation with X-rays diminishes for a time an animal's capacity to form antibodies to bacterial antigens and heterologous erythrocytes (Hektoen, 1915, 1918, 1920; Murphy and Sturm, 1925; Craddock and Lawrence, 1948), and also, at least in the rat, diminishes the animal's resistance to heterologous tumour transplants (Murphy, 1914b, 1926).

It seemed likely, therefore, that previous exposure of the host to ionizing radiation would result in prolonged survival of homotransplants, and Dempster, Lennox and Boag (1950) showed that this was in fact the case. Working with rabbits they found that, following total body irradiation to a dose of 250 r the day before grafting, the period of survival of skin homografts was increased, in one case up to 24 days, and hyperplastic proliferation of the cells of the epidermis, which is characteristic of autotransplants but normally absent or insignificant in homotransplants, was quite marked from the 4th to about the 16th day. The destructive process could not be held in check indefinitely, however, and after a period of quiescence relatively rapid breakdown ensued in every case. Moreover, when second-set grafts were made from the same donor about a week after those of the first set had

been destroyed their survival time was not prolonged by irradiation given either before the first set or immediately after the second set

Irradiation in the dose used produced a marked lymphopenia but the second set transplants were destroyed in spite of irradiation enough to maintain a low level of circulating lymphocytes and to damage lymphoid tissue throughout the body and Dempster *et al* concluded somewhat hastily that the lymphocytes which have so often been ascribed a major role in the death of foreign epithelium or transplanted tumour cannot have any part in the actual destruction of the graft

Later Dempster (1953a) reported that the *survival of kidney homotransplants in dogs* was not prolonged by prior irradiation of the host and this was confirmed by Baker and Gordon (1955) using a dose of 225 r which was lethal to about 50 per cent of dogs and reduced the leucocyte count in surviving animals to 500/c mm or less

These findings were not encouraging but the discovery that animals could recover after otherwise lethal irradiation if they were given transplants of haemopoietic tissue opened up new and exciting possibilities

As a rule death following total body irradiation to or a little beyond the minimum lethal dose is the result of damage to either the haemopoietic tissues or the gut Damage to the haemopoietic tissues results in anaemia polymorphonuclear leucopenia lymphopenia and thrombocytopenia and death may occur from infection or haemorrhage while damage to the gut is manifested by increased permeability of the wall to microorganisms and consequent peritonitis Which factor predominates depends on the species of animal concerned and on the physical characteristics of the radiation In mice damage to haemopoietic tissue is normally the limiting factor With rats when the usual type of apparatus generating X rays at 70 to 200 kv is used death is likely to occur from peritonitis and administration of streptomycin and other antibiotics though helpful may fail to prevent this Anderson Delorme and Woodruff (unpublished) found however that hooded rats exposed to 1000 r from a supervoltage therapy machine operating at 4 MeV died regularly as a result of damage to haemopoietic tissue and not from peritonitis

The protective effect of transplanting haemopoietic tissue was at first attributed to some chemical agent liberated by the transplanted cells but it has now been shown that these cells may survive and multiply and it seems likely that this accounts for the protection The evidence will be *considered in detail in Chapter 22* but may conveniently be summarized here In the first place following irradiation and homotransplantation of haemopoietic tissue erythrocytes antigenically like donor erythrocytes have been found in the peripheral blood (Lindsley Odell and Tausche 1955 Odell Tausche Lindsley and Owen 1957) secondly cells capable of evoking immunity to homotransplants of donor strain tissue have been demonstrated in the spleen lymph nodes and peritoneal exudate (Mitchison 1956) and finally cells recognizable cytologically as being of donor origin have been found in the bone marrow (Ford Hamerton Barnes and Loutit 1956)

Animals in which foreign cells continue to thrive are called *chimeras* and when this state of affairs results from irradiation and transplantation they are called *irradiation chimeras*

Homologous cells are less effective than isologous cells in the sense that many more are required to give protection Moreover even when the [illegible] homologous cells is sufficient [illegible] immediate recovery the recipient [illegible] one to two months later [illegible] and diarrhoea and there [illegible] evidence that this is due

to the transplanted cells reacting immunologically against the host (p. 157).

Animals may also recover after a lethal dose of irradiation if they are joined in parabiosis with non-irradiated partners (Binhammer, Schneider and Finerty, 1953; Finerty, Binhammer and Schneider, 1953; Schneider, Wybourn, Binhammer and Finerty, 1954). In the experiments cited the animals were joined to litter mates, and were usually left in parabiosis for 30 days, but some survived after being in parabiosis for only 4 days. It made no difference to the results if the partners were splenectomized, adrenalectomized or hypophysectomized.

It seems likely that the protection resulting from parabiosis depends on the transfer of haemopoietic cells from the non-irradiated partner, but so far no attempt appears to have been made to determine whether or not the irradiated animals become chimeras.

It is natural to ask whether a radiation chimera will accept homotransplants of other tissues from the donor of the haemopoietic tissue or an animal isogenic with it, but it will be convenient to postpone consideration of this question until we come to discuss the phenomenon of specific immunological tolerance (Chapter 7).

Administration of Chemical Cytotoxins

In the search for chemotherapeutic agents effective for the treatment of neoplastic disease (Chapter 11), a number of compounds have been found which cause widespread damage to haemopoietic tissue resembling in many ways the damage caused by irradiation.

Baker, Gordon, Huffer and Miller (1952) studied the effect of one compound of this kind, nitrogen mustard, on the behaviour of kidney homotransplants in dogs, but found that even when the dose was sufficient to cause a marked leucopenia survival of the transplants was not prolonged. Similarly Ingram and Woodruff (unpublished) were unable to prolong the survival of skin homografts in mice by administration of a folic acid analogue, aminopterin.

More recently however it has been shown that an animal may recover following administration of a lethal dose of Myleran (p. 161) if given homologous bone marrow, and it seems likely that this protective effect depends on survival of the transplanted cells, at any rate until the host's bone marrow has time to recover.

Administration of Cytotoxic Sera

It was shown by Chew and Lawrence (1937) that a serum having a powerful antilymphocytic effect *in vivo* can be prepared by immunizing the animal with injections of suspensions of heterologous lymphocytes, and this observation has been confirmed by Cruickshank (1941). Somewhat similar sera, usually referred to as "cytotoxic antireticular sera" (Straus, 1946) have been prepared using as antigen a mixture of minced heterologous spleen, lymph node and bone marrow.

It seemed possible that by administering such sera at regular intervals to the recipient of a homotransplant some information concerning the role of the lymphocyte in relation to destruction of the transplant might be obtained. Antilymphocytic serum (rabbit-anti-rat) was accordingly prepared by injecting rabbits subcutaneously with a suspension of lymphocytes obtained by grinding up rat lymph nodes. The injections were given daily for seven days, the total lymphoid tissue obtained from one rat being divided each day between two rabbits, and after a further eight days the rabbits were bled by cardiac puncture. The serum was separated and heated to 56°C. for an hour in a water bath to diminish anticomplementary activity, and absorption was carried out against rat erythrocytes until no agglutinins remained and haemolysins could not be detected beyond a dilution of one in four.

TABLE I

THE EFFECT OF ANTILYMPHOCYTIC SERUM ON THE LEUCOCYTE COUNT IN THE PERIPHERAL BLOOD OF RATS

(Woodruff, Woodruff and Forman, unpublished data)

	Time after Injection												
	0 hours	*2 hours*	*8 hours*	*24 hours*	*2 days*	*3 days*	*4 days*	*5 days*	*6 days*	*7 days*	*8 days*	*9 days*	*10 days*
Lymphocytes per c mm	9,130	920	1,6[illegible]0	4,290	3,020	1,720	2,650	2,480	4,700	3,310	3,7[illegible]0	1,310	9,100
Polymorphs per c mm	3,370	1,330	7,830	2,210	4,730	2,560	2,5[illegible]0	3,720	3,800	2,850	2,500	2,600	4,900

To test the antilymphocytic activity of the serum adult Wistar rats* were given 1.0 ml daily by intraperitoneal injection. Total and differential leucocyte counts were performed frequently during the first 24 hours, and thereafter once daily just before each injection, and from the figures obtained the absolute polymorphonuclear and lymphocyte counts were calculated. A typical series of counts is shown in Table I.

It will be seen that the lymphocyte count rose abruptly between the ninth and tenth days, and a similar rise occurred about the same time in every case. The explanation of this phenomenon is uncertain, but it has been suggested that the recipient animal develops anti-antilymphocytic antibodies which render the serum ineffective. Whatever the explanation it seemed unlikely, in view of the relatively short time for which the lymphocyte count could be held at a low level, that the behaviour of a homotransplant—at any rate of normal adult tissue—would be greatly modified by administering antilymphocytic serum to the recipient, and experiments with transplants of skin, ovary and adrenal* from hooded rats to Wistars confirmed this prediction (Woodruff, Woodruff and Forman, unpublished).

*Rats destined to receive the serum were given 6.0 mg neoarsphenamine intramuscularly because it was found that animals not so treated frequently developed a severe anaemia after repeated injections, almost certainly because they were harbouring the parasite *Bartonella muris*. This anaemia appears comparable to that known to follow splenectomy (Marmorston-Gottesman and Perla, 1930) in *Bartonella* infected rats.

Reticulo-endothelial Blockade

Blockade of the reticulo-endothelial system may be produced by injecting various colloidal suspensions including Indian ink, dyes such as trypan blue and congo red, and thorotrast.

This procedure has been widely used in experiments designed to determine the site of antibody formation and, despite some conflicting reports, there is convincing evidence that, in rabbits, reticulo-endothelial blockade diminishes the animal's capacity to form antibodies to bacterial antigens, heterologous erythrocytes and heterologous serum (see Roberts, 1929, Cannon, Baer, Sullivan and Webster, 1929, Jungeblut, 1930, Jaffe, 1931, Ehrlich and Harris, 1945, McMaster, 1953). Two explanations have been suggested to account for this phenome-

* The serum was given intraperitoneally in a dose of 1 ml daily. Recipients of ovarian and adrenal transplants had the corresponding glands removed. It was found that while adrenalectomy and injection of antilymphocytic serum were each well tolerated the combination was usually rapidly fatal.

[illegible] According to the older view the blockade diminishes the capacity of the affected cells to form antibody in the presence of antigen; according to the more modern view the blockade interferes with the capture and *preparation of antigen* (McMaster, 1953).

It would seem likely, in view of the findings quoted above, that the survival of [illegible] grafts would be prolonged in hosts [illegible] to reticulo-endothelial blockade. [illegible] of the investigations which have [illegible] confirm this prediction. Thus [illegible] and Kreuzwendedich von dem [illegible] [illegible] Urbach and Schnitzler (1928), [illegible] and Ludford (1932) found an [illegible] susceptibility to tumour trans[illegible] mice subjected to blockade, and [illegible] and Tammann (1925, 1926) and [illegible] 1931) found that the survival of [illegible] grafts, in mice and guinea pigs [illegible] is prolonged in blockaded [illegible] [illegible] and Appel 1933) made the [illegible] remarkable observation that rabbits [illegible] *immune to the Brown Pearce* [illegible] times became susceptible again [illegible] daily intravenous injection [illegible]

On [illegible] negative findings have been [illegible] Munck (1928) and Kaliss and B[illegible] * in respect of tumour homot[illegible] in mice, and by Loeb (1945) [illegible] of skin homografts in rabbits. [illegible] that these discrepancies depend [illegible] in the thoroughness of the [illegible] and in the time at which it was [illegible] relation to the time of transplant[illegible] further investigation is necessary [illegible] the matter.

Splenectomy

The spleen, though not indispensable, appears to play a significant part in the development of immunity to infections. Moreover, as Rowley (1950a, b) has shown in both man and the rat, the formation of antibodies following intravenous injection of heterologous *(sheep) erythrocytes is impaired in* splenectomized individuals. Nevertheless, despite the contrary claim of Apolant (1911c), splenectomy appears to have no consistent effect on the behaviour of subsequent homotransplants of tumours (*see* Woglom, 1929 for review) or, at any rate in rabbits, on the behaviour of homografts of skin (Krohn, 1953, 1954d).

Lymphadenectomy

Billingham, Brent and Medawar (1954b) excised the ipsilateral axillary lymph nodes from mice *immediately* before transplanting *homologous skin to the side of the chest,* but found that the survival of the grafts was only very slightly prolonged.

In another experiment they excised (or destroyed chemically) primary skin homografts after they had been 11 days *in situ,* and removed the regional lymph nodes at the same time. Nineteen days later (i.e. 30 days after the transplantation of the first graft) each animal received a second graft from the same donor strain to the opposite side of the chest, and these behaved like normal second grafts in control hosts which had not been subjected to lymphadenectomy.

The first of these experiments is easily explained by the opening up of new lymphatic pathways. The second clearly implies that 11 days after skin grafting the regional nodes are not uniquely responsible for the state of immunity which exists in the host, but it is conceivable that they might be at an earlier stage. Experiments could easily be designed to investigate this and might prove illuminating.

* Kaliss and Bryant found however that administration of trypan blue increased the enhancing effect (p. 131) of [illegible] tumour

Administration of Corticosteroids and ACTH

General Biological Properties of Corticosteroids and ACTH

A distinction must be drawn between administration of corticosteroids (i.e. steroids which are either secreted by the adrenal cortex or have biological properties resembling those of the steroids secreted by the adrenal cortex), and administration of the pituitary hormone ACTH. ACTH causes hypertrophy of the adrenal cortex and stimulates the secretion of a variety of steroids, whereas administration of corticosteroids inhibits secretion by, and if long continued causes atrophic changes in, the adrenal cortex.

It is fortunately not necessary to review here the vast literature concerning the biological properties of corticosteroids and ACTH, or the clinical use of these hormones in the treatment of rheumatoid arthritis, rheumatic fever, allergic disorders, diseases of the blood and the eye, and the so-called collagen diseases such as disseminated lupus erythematosus and periarteritis nodosa. The following facts are relevant, however, either because they first suggested that it would be of interest to study the effect of administration of corticosteroids and ACTH on homotransplants, or because they help us to assess the significance of the results of such experiments.

Effect on the Blood Leucocyte Count. Following the injection of an effective dose of cortisone or ACTH there is, within a few hours, a sharp fall in the number of circulating lymphocytes (Hills, Forsham and Finch, 1948; confirmed by many workers in man and laboratory animals), and an even more dramatic fall in the eosinophil count.

Effect on Wound Healing. Administration of cortisone or ACTH inhibits the formation of granulation tissue and fibroblastic proliferation in general. It thus delays wound healing especially when there is a large defect which can heal only by granulation and re-epithelialization from the edge (Ragan, Howes, Plotz, Meyer and Blunt, 1949; Howes, Plotz, Blunt and Ragan, 1950; Spain, Molomut and Haber, 1950; Baxter, Schiller, Whiteside and Straith, 1951; Baxter, Schiller and Whiteside, 1951; Bangham, 1951; and many others). The effect is less noticeable, and sometimes not demonstrable (Cole, Orbison, Holden, Hancock and Lindsay, 1951), in the case of clean incised wounds which heal by first intention.

Effect on Immunological Phenomenon. It has been shown that administration of cortisone or ACTH in adequate dosage, starting prior to, or at the same time as, the first injection of an appropriate antigen, inhibits the formation of antibodies (Germuth and Ottinger, 1950), the development of hypersensitivity (Germuth and Ottinger, 1950), the Arthus phenomenon (Germuth and Ottinger, 1950; Seifter, Ehrich, Begany and Warren, 1950; Germuth, Nedzel, Ottinger and Oyama, 1951), and the Shwartzman phenomenon (Soffer, Shwartzman, Schneierson and Gabrilove, 1950; Thomas and Mogabgab, 1950) in rabbits; and the development of experimental encephalitis in guinea pigs (Moyer, Jervis, Black, Koprowski and Cox, 1950). On the other hand, according to Mirick (1950), it does not inhibit the formation of antibodies to pneumococcal polysaccharides in man. Administered with a drug to which a patient has previously reacted violently cortisone may prevent the development of manifestations of drug hypersensitivity (Goldman and Rockwell, 1951).

Administration of cortisone and ACTH to previously immunized animals may, or may not, cause a change in the level of circulating antibodies.

Bjorneboe, Fischel and Stoerk (1951) found that injection of these hormones to

rabbits immunized against pneumococcal polysaccharides caused a fall in circulating antibody level.

Dougherty, Chase and White described a different phenomenon (Dougherty, Chase and White, 1945; Chase, White and Dougherty, 1946). They reported that if animals were immunized and then kept until the antibody titre had fallen to a low level, the injection of adrenal cortical extract or ACTH resulted in a sudden rise in titre. This so-called anamnaestic response was attributed to the release of antibody from lymphocytes, destroyed as a consequence of the injection. Many other workers have since performed similar experiments, however and have found no change in antibody level (Eisen, Mayer, Moore, Tarr and Stoerk, 1947, Fischel, Le May and Kabat, 1949 Herbert and De Vries, 1949; De Vries, 1949 [illegible] and Timiras, 1951)

Administration of cortisone or ACTH to appropriately sensitized animals or human subjects [illegible] not prevent either the development [illegible] hypersensitivity reactions of the [illegible] type (Harris and Harris, 1950 [illegible] and Friedlaender, 1950; Stoller[illegible] and Plotz, 1951), the Arthus [illegible] (Harris and Harris, 1950 [illegible] 1950), or the Shwartzman phenomenon [illegible], Shwartzman, Schneierson and [illegible], 195[illegible] in response to further injections of the antigen It does however prevent the development of fatal anaphylactic [illegible] in sensitized mice following [illegible] injection of a challenging dose of [illegible] (Nelson, Fox and Freeman, 1950) [illegible] according to Hoene, Coutu, Horava, Procopio, Robert and Salgado (1952), it does so also in guinea pigs if given in sufficiently large dosage, despite previous reports to the contrary (Leger, Leith and Rose, 1948, Harris and Harris, 1950; Friedlaender and Friedlaender, 1950, Malkiel, 1950 Dworetzky, Code and Higgins, 1950, Landau, Nelson and Gay, 1951)

The lack of effect on skin hypersensitivity reactions of the immediate type accords with the observation (Friedlaender and Friedlaender, 1950; Long and Favour, 1950) that administration of cortisone and ACTH does not modify the skin reaction to an intradermal injection of histamine. The prevention of anaphylactic shock is more difficult to understand in view of the fact that these hormones do not protect a guinea pig against a lethal dose of histamine (Landau, Nelson and Gay, 1951).

Hypersensitivity of the delayed type, unlike immediate-type hypersensitivity, is suppressed by cortisone and ACTH in previously sensitized rabbits, guinea pigs* and human subjects (Harris and Harris, 1950; Long and Favour, 1950; Osgood and Favour, 1951) It has also been reported (Hensler, Wurl and Gillespie, 1952) that penicillin sensitivity reactions may be temporarily abolished by cortisone or ACTH.

Cortisone and ACTH do not prevent the passive Arthus phenomenon, produced by intracutaneous injection of antigen and antibody, in rabbits (Germuth and Ottinger, 1950), nor the passive transfer of skin-sensitizing antibodies by serum (the Prausnitz-Kustner reaction) in man (Stollerman, Rubin and Plotz, 1951).

Effect of Corticosteroids and ACTH on Homotransplants

Skin Grafts. Soon after cortisone and ACTH became available for clinical use there were dramatic reports of the effect of these hormones in prolonging the survival of skin homografts in man, and permanent survival was claimed in some cases (e.g. Whitelaw, 1951) Further investigation has failed to confirm these findings, and it now appears that, in the dosage ordinarily employed, these hormones have no decisive effect on the survival of skin homografts in man (Ellison, Martin, Williams, Clatwor-

*Sheldon Cummings and Evans (1950) failed to suppress the tuberculin reaction in guinea pigs with active tuberculosis but this may have been because they used such a small daily dose of cortisone (5 mg or less)

thy Hamwi and Zollinger 1951 Weisman Quimby Wright and Cannon 1951 Baxter, Schiller Whiteside Lipshutz and Straith, 1951 May Oakey and Pilling 1952)

In rabbits on the other hand Billingham *Krohn and Medawar* (1951a) found that the reaction to homografts could be modified and the period of survival approximately trebled by administering cortisone in a dosage of 10 mg daily though the grafts were *always* destroyed by the 25th day despite continuance of the treatment Attachment vascularization and differentiation of control autografts were delayed and mitotic activity was greatly reduced

Further experiments have greatly extended these findings and have confirmed that the results differ in different species

In rabbits cortisol* (Krohn 1954b) halocortisols (Woodruff and *Elhurado* 1955 1956) and prednisone (Woodruff and Elhurado 1955 1956) administered systemically are at least as effective as cortisone in prolonging the survival of first set homografts (Fig 31) but as Krohn (1954b) has shown desoxycorticosterone progesterone testosterone and oestradiol are ineffective Cortisone acetate (Billingham Krohn and Medawar 1951b) and *one of the halocortisols* (Woodruff and Elhurado 1955 1956) have also been shown to be effective on local application provided that in hosts bearing several grafts each one is treated On the other hand ACTH is ineffective in rabbits (Allen Williams Lovingood and Ellison 1952 *Krohn* 1954a) unless as Krohn (1956) has shown it is administered for about a month before grafting and continued thereafter while the grafts are in place

Cortisone has no effect on second set homografts in rabbits immunized by a first graft from the same donor unless it is administered for about a month prior to grafting (Krohn 1955c)

In guinea pigs Weisman *et al* (1954) reported negative results with both cortisone and ACTH but subsequently Sparrow (1953) showed that the period of survival of skin homografts may be at least doubled by giving cortisone in sufficiently large dosage (25 mg daily) More recently Sparrow (1954) has shown that ACTH administered in a slow absorption medium in a dose of 12 5 mg daily is even more effective

In mice Conway Stark and Joslin (1952) using the transparent chamber technique found that ACTH had no decisive effect on skin homograft survival but cortisone according to Medawar (1954) prolonged survival if given in a dose of 0 5 mg daily although in larger dosage it sometimes actually shortened the period of survival by causing ischaemic mummification of the grafts More recent experiments (Medawar *and Sparrow* 1956) have shown that cortisone (0 1 mg daily) and ACTH (1 mg daily) *in a slow absorption medium* are both effective and corticosterone has a just perceptible cortisone like action but desoxycorticosterone progesterone testosterone and oestradiol are ineffective

In monkeys (*Macaca mulatta*) the survival of skin homografts is prolonged to only a trivial extent by daily administration of either 15 mg cortisone or 30 mg ACTH in a slow absorption medium (Krohn 1955c)

In pigs ACTH has been found to be ineffective (Weisman *et al* 1951) In chickens according to Cannon and Longmire (1952) cortisone is effective but ACTH is not

Homotransplants of Whole Kidneys Persky and Jacob (1951) found that neither cortisone (215 mg daily) *nor* ACTH (100 mg daily) prolonged the life of homotransplants of whole kidneys by vascular anastomosis in dogs

The failure of cortisone to prolong the survival of kidney homotransplants in dogs was confirmed by Dempster (1953c) and De Klerk Scott and Scott (1954) although

*[illegible] are different names for the same substance



Fig. 31 Effect of steroids on the survival of skin homografts in rabbits. A Biopsy of graft after 8 days in control recipient. Breakdown is just complete. B, C, D Biopsy of graft after 8, 12 and 16 days respectively in recipient given fluorohydrocortisone systemically (0.3 mg/kg body weight daily). At 8 days and 12 days the graft shows epithelial hyperplasia and there is little evidence of host reaction, but by 16 days breakdown is almost complete. E, F Biopsy of graft treated by local application of fluorohydrocortisone after 8 and 16 days respectively. There is little cellular reaction. Epithelial hyperplasia is slight but there is much desquamation. Breakdown was not complete until 20 days. Haematoxylin and eosin. ×160.

Dempster found that the histological changes in the transplant were modified and that in the early stages the transplants in cortisone treated hosts secreted more urine than those in the controls. On the other hand Baker, Gordon, Huffer and Miller (1952) reported that kidney homotransplants functioned on average for 13.5 days in dogs treated with both cortisone (50 mg daily) and nitrogen mustard, compared with 3 days in untreated dogs and 1.5 days in dogs which received nitrogen mustard but no cortisone, and the difference in the cortisone treated hosts appeared to be statistically significant.

Free Endocrine Transplants. Woodruff and Boswell (1953) studied the effect of cortisone and ACTH on transplants of both immature and adult adrenal in rats. It seemed likely that cortisone, whatever its effect on the development of immunity, might prove deleterious to the transplants, since atrophy of the adrenals occurs in normal rats treated with cortisone for some weeks (Winter, Silber and Stoerk, 1950), probably owing to inhibition of the animal's own secretion of ACTH. To guard against errors due to this cause some animals received large doses of cortisone and others small doses, and all animals which received cortisone received also ACTH. Furthermore, whenever possible the behaviour of homotransplants was compared with that of autotransplants in animals receiving the same treatment.

It was found that prolonged administration of cortisone in a dosage of 10 mg daily was not well tolerated and exerted a deleterious influence on both auto- and homotransplants. Cortisone in a dosage of 2 mg daily, given with 7.5 mg ACTH, was well tolerated and did not impair the regeneration of autotransplants. Neither this combination of hormones nor ACTH alone however, had any decisively beneficial effect on the survival of homotransplants.

In a somewhat similar investigation in man, the author (Woodruff, 1953a) administered cortisone and ACTH combined in various dosages to four patients with Addison's disease who received free transplants of human foetal adrenal. The patients showed dramatic improvement which lasted for several months after withdrawal of the hormones, but subsequent biopsy revealed no trace of the transplants.

The author has also studied the effect of cortisone and thyrotrophin on thyroid transplants in guinea pigs (Woodruff, 1953c, 1954a). Again it was felt that cortisone might prove directly harmful to the transplants (cf. Perry, 1951, Paschkis, Epstein, Cantarow and Friedler, 1952), and similar precautions to those used in the experiments with rat adrenal were employed. It was found that cortisone in large dosage (20 mg daily) decreased the chance of survival of autografts even when thyrotrophin was administered simultaneously; it nevertheless appeared to increase slightly the chance of survival of homografts. Cortisone in small dosage (3 mg daily) had no apparent effect on either autotransplants or homotransplants.

Corneal Transplants. As we have seen, Maumenee (1951) found that corneal transplants in rabbits could be rendered cloudy by making homotransplants of skin from the same donors two weeks after the corneal transplantation. He showed further that administration of cortisone systemically in large dosage (15 mg per kg body weight per day), starting within two days of the commencement of clouding, caused the transplants to become clear again.

Blood Vessel Transplants. The functional value of blood vessel homotransplants depends as a rule on their being replaced, at least in part, by host tissue. It is not surprising therefore that cortisone has been found (Barberio, Pate, Sawyer and Hufnagel, 19[illegible]) to be of little or no benefit, and

sometimes actually harmful, when administered to recipients of aortic homotransplants.

Embryonic Tissues. Toolan (1957a) found that homografts of embryo skin, gut and stomach transplanted to the chest wall survived indefinitely in cortisone treated rabbits and rats, but not in untreated animals.

Tumour Homotransplants. Cortisone appears to facilitate the survival and metastasis of transplantable tumours. Stoerk, Budzilovitch and Bielinski (1952) showed that rats normally susceptible to a transplantable lymphosarcoma became resistant if injected with homologous normal lymphoid cells; resistance did not develop, however, if cortisone was administered for two to three weeks, starting at the time of injection. Later Stoerk (1953a, c) reported that resistance developed after *repeated* injections of suspensions of liver or kidney from normal but not from cortisone treated, rats; the significance of the observation is, however, obscure.

[illegible] (1953d) has reported further that [illegible] resistance to tumour transplants [illegible] be suppressed by administration [illegible] and Pomeroy (1954) found that [illegible] was given concomitantly with [illegible] intravenous injection of a transplantable [illegible] carcinoma to Swiss mice, widespread metastasis developed beyond the lung filter.

Gasic, Hoecker, Pizarro and Baydak (1957) made the interesting observation that if mice immunized against a transplantable leukaemia were given a course of injections of cortisone (6-8 daily injections starting 7-19 days after the second immunizing dose of leukaemic cells), it was no longer possible to transfer immunity by adoptive immunization to another animal of the host strain.

Conclusions. Cortisone, given in appropriate dosage, prolongs the life of skin homografts in rabbits, guinea pigs and mice, but not to any appreciable extent in dogs, monkeys or humans. *Cortisol* appears to be at least as effective as cortisone, but corticosterone has but little effect. Cortisone also prolongs slightly the life of thyroid homografts in guinea pigs, and facilitates the survival and metastasis of tumour homotransplants in rats and mice. ACTH prolongs the survival of skin homografts in guinea pigs and mice, but not in rabbits, monkeys, chickens or humans.

The species differences have not been fully explained. It was shown by Bush (1953) that most of the hormone produced by the rabbit's adrenal is corticosterone, and as Krohn (1954b) has pointed out this suffices to explain why ACTH has so little effect on skin homografts in rabbits. The ineffectiveness of both cortisone and ACTH in primates suggests however that species may differ also in respect of the sensitivity of their reactive tissues to cortisone (Krohn, 1955).

The mode of action of cortisone is also far from clear. As Scothorne (1956) has suggested it almost certainly interferes with the liberation of antigens from grafts, but it seems no less certain that there is a central interference (p. 134) with the mechanism of immunological response, and there may also be some interference with the effector mechanism (p. 95).

Administration of Antihistamine Drugs

Foster and Hanrahan (1948) suggested that histamine might be liberated as the result of an antigen-antibody reaction between graft and host, and might be partly responsible for destruction of a homograft. To test this hypothesis they studied the effect of an antihistamine drug, pyribenzamine, on the survival of a split skin homograft in a human subject. The donor was a white male, the recipient a coloured female, and the drug was administered for 60 days. The graft was observed for 90 days

and appeared healthy throughout this period. The authors wisely refrained from drawing any firm conclusions from this one case and themselves suggested that replacement of the graft by host tissue may have been mistaken for survival. This appears to have been the first investigation of its kind, though the suggestion that antihistamines might prolong the survival of homografts had been made previously by Hamburger (1917).

Marconi (1950) studied the effect of another antihistamine drug, Neo-antergan (N-dimethylamine ethyl-N-methoxyaminopyridine), on homografts and heterografts of skin in guinea pigs. Both the homografts and the heterografts appeared to survive slightly longer in treated hosts than in the untreated controls, but the difference was slight and was not regarded by the author as significant. These experiments are open to criticism, however, firstly because the drug, which is fairly short-acting, was given only twice daily, and secondly because the state of the grafts was judged on their macroscopic appearance only.

In view of these rather inconclusive findings, and the theoretical and practical importance of the matter, Woodruff and Boswell (1951) conducted further experiments using a powerful and relatively long-acting drug, Phenergan (promethazine hydrochloride). The animals used were adult guinea pigs, and each host received either orthotopic skin grafts or free subfascial grafts of thyroid.* Both autografts and homografts were studied. Phenergan was administered thrice every 24 hours, in a total daily dosage of 15, 25 or 60 mg. per kg. body weight.

It was found that the drug had no decisive effect on the behaviour of either the autografts or the homografts†. Before these findings were published further essentially negative results were reported by Conway, Jerome and Stark (1953), who studied the effect of oral administration of Benadryl (diphenhydramine hydrochloride) on skin homografts in mice.

These experiments confirm Marconi's findings, but they do not necessarily support his conclusion that histamine liberation is not an important factor in homograft destruction. For, as Dale (1918) has pointed out, antihistamine drugs may be ineffective when the cells which liberate histamine themselves react to it (the "intrinsic" type of reaction), and the same may be true when histamine is liberated in the immediate vicinity of the reacting cells.

It would be of interest to repeat these experiments in rabbits, because the Arthus phenomenon, which resembles in many respects the homograft reaction in this species (Rogers, 1950), is known to be inhibited by Phenergan (Benacerraf and Fischer, 1949). Meanwhile, however, the observed results provide further evidence of similarity between the homograft reaction and delayed-type bacterial sensitivity, which likewise is not affected by antihistamine drugs.

Administration of Anticoagulants

Conway, Stark and Joslin (1953a), having observed that thrombosis in small blood vessels was associated with, and appeared to play an important part in bringing about, the destruction of skin homografts in mice, decided to test the effect of administering an anticoagulant, dicoumarol, to homograft recipients. The drug was administered by mouth, starting 2-4 days before grafting, and the grafts were observed by means of the transparent chamber technique.

It was found that thrombosis could be delayed and sometimes prevented in this way, but capillaries failed to invade the

* The recipients of thyroid grafts were all thyroidectomized.

† The proportion of surviving thyroid homografts after four weeks and again after four months was slightly greater in the treated animals than in the controls but the difference was probably not significant.

graft and the period of survival was not greatly prolonged.

Administration of Substances Which Lower the Titre of Properdin

It has been shown by Pillemer and his colleagues (Pillemer, 1956; Wardlaw and Pillemer, 1956) that normal human and other mammalian sera contain a beta-euglobulin, *properdin*, which in the presence of magnesium ions and complement kills, lyses or inactivates certain bacteria, protozoa, abnormal erythrocytes, phage and viruses, in the absence of specific antibodies.

The titre of properdin in serum is depressed by total body irradiation; experimental haemorrhagic shock; and administration of zymosan, dextrans, levans and certain other carbohydrate complexes

In the light of these facts Hubay and Persky (1957) compared the behaviour of kidney homotransplants to the carotid-jugular system in normal dogs and dogs whose serum properdin titre had been lowered by an intravenous injection of zymosan (5 10 mg /kg)

Two animals died from septicaemia despite post operative administration of penicillin and streptomycin, and even animals which received neomycin before operation as an additional precaution sometimes developed infection in the neck. In spite of this evidence of the effect of the zymosan on the animals' resistance to bacterial infection and the fact that the properdin titre remained depressed for 2-3 weeks, the period of survival of the homografts in the treated animals ranged from 1 to 8 days and did not differ significantly from that observed in the controls.

It seems desirable however, as Hubay and Persky have suggested, that further investigations should be undertaken before any general conclusions are drawn concerning the role, if any, of the properdin system in homograft destruction.

Thyroidectomy and Hypophysectomy

Molomut (1939) found in rats that hypophysectomy had no effect on the capacity to form antibodies, and in view of the relationship of the pituitary to other endocrine glands he has suggested that no endocrine lesion is likely to diminish antibody formation.

Nevertheless it seemed worthwhile, for two reasons, to study the effect of thyroidectomy on the behaviour of skin homografts. In the first place it has been found that thyroid homotransplants survive longer than skin homografts in the only species (the guinea pig) in which the comparison has been made (Woodruff and Woodruff, 1950; Woodruff, 1953b; Woodruff, 1954a; Woodruff and Boswell, 1954). This might be due to differences in dosage or in the properties of the two tissues, but it seemed possible that it might result from the fact that the thyroid recipients, unlike the skin recipients, were thyroidectomized. Secondly, administration of cortisone, which we have seen (p. 105) prolongs the life of skin homografts greatly in rabbits and to a less extent in guinea pigs, is known to depress thyroid function (Perry, 1951; Paschkis, Epstein, Cantarow and Friedler, 1952).

It would seem desirable to perform experiments in both rabbits and guinea pigs, but so far the experiments have been confined to rabbits In this species it has been found (Woodruff, 1954b) that skin homografts and autografts behave similarly in thyroidectomized and normal hosts. In some experiments thyroidectomy was performed one week prior to skin grafting, in others eight weeks* prior to skin grafting, but the results were the same in both cases.

Krohn (1955a) administered thiouracil to guinea pigs, in a dosage sufficient to cause great enlargement of the thyroid gland, but

*It was thought desirable to include animals thyroidectomized so long in advance because, as Hornabrook (1953) has shown, some of the effects of thyroidectomy, at least in rats, do not become manifest for some weeks.

found that this procedure also did not modify the behaviour of skin homografts

Schatten and Bergenstal (1958), on the other hand, found that the mean survival of skin homografts was 10.5 ± 0.14 days in hypophysectomized rats, and 10.0 ± 0.38 days in rats made thyroid deficient by radioactive iodine, as compared with 7.1 ± 0.38 days in normal rats (*see also* Schatten, Bergenstal, Kramer and Wexler, 1958)

The reason for the difference between these findings is not clear Woodruff used histological criteria to determine the end point of survival, while Schatten and Bergenstal relied on gross appearance, but this alone should not account for the discrepancy and it may be, as Schatten and Bergenstal have suggested, that the effect of hypothyroidism on homograft survival may differ in different species

Alteration of the Body Temperature

In cold blooded animals it is possible to study the effect of altered body temperature by altering the temperature of the environment Hildemann (1956) carried out experiments of this kind in goldfish and found that the survival time of scale homografts was greatly reduced by increasing the environmental temperature Thus at 15°C autografts and homografts were indistinguishable for several days, whereas at 28°C the homograft reaction was so rapid that there was conspicuous haemorrhage in the recipient contact zones within 24 hours of grafting, and vascularization occurred in only a few grafts and lasted at most for a day or two

PROCEDURES AFFECTING PRIMARILY THE TRANSPLANT

Attempts to obtain long survival by altering the capacity of the transplant to evoke a reaction, or to survive in the face of a given reaction, have for the most part been unsuccessful, despite some exaggerated claims to the contrary

The following methods have been tried

1 "Activation" of the transplant before removing it from the host

2 Exposure of the transplant to the action of heat, cold, X rays, chemicals, and enzymes

3 *Culture of the tissue in vitro* prior to transplantation.

4 Preliminary heterotopic autotransplantation

5 *Hormonal stimulation of the transplant in the host*

Activation of the Transplant

Silberberg (1934) administered pituitary extract, which was apparently rich in thyrotrophin, to guinea pigs, and later transplanted the hypertrophic thyroid from these animals to other guinea pigs He found, however, that these transplants were destroyed more rapidly than homotransplants from untreated donors

Rous (1916) rendered rabbit skin hypertrophic by various agents, and then transplanted the hypertrophic skin autologously He found that these grafts united to the bed more rapidly, and were vascularized sooner, than normal grafts The stimulated epithelium proliferated almost at once and quickly covered adjacent raw surfaces On the other hand, in rodents skin in an active phase of the hair cycle has long been considered unsatisfactory for homografting, and Randall and Dushoff (1958) have now shown that the survival of homografts of such skin in mice is appreciably shorter than that of homografts of inactive skin.

Treatment with Physical and Chemical Agents

Blumenthal (1941) reported that the lymphocytosis which normally occurs in homograft recipients does not occur if, *prior to* transplantation, the graft is heated to 56°C. or treated with reagents which denature proteins. These procedures destroy the T antigens (p. 93), but in addition kill the cells, so that the graft cannot be said to survive at all.

Irradiation of a whole-kidney prior to homotransplantation by vascular anastomosis does not alter the period of survival, but does alter the histological picture (Dempster, *1953a*). Kidneys *so treated show no* plasma cell reaction, and from this Dempster has concluded that the plasma cells which appear in large numbers in normal kidney transplants originate in the transplants themselves and not in the host.

It is possible however, according to Dukes and Blocker (1952), to prolong the life of skin homografts about 60 per cent by treating them, prior to grafting, with a mixture of streptokinase and streptodornase, but the significance of this observation is obscure.

Cultivation *In Vitro* Prior to Transplantation

It has been claimed that tissue cultured *in vitro in an appropriate medium may* survive homotransplantation longer than tissue taken directly from the donor.

Stone, Owings and Gey (*1934*a, b) reported some years ago that they had successfully treated a patient with parathyroid tetany by means of homotransplants prepared in this way. Subsequently Lux, Higgins and Mann (1937a, b) studied the behaviour of homotransplants of adrenal tissue which had been grown in culture in rabbits, guinea pigs and rats, but the results obtained were too anomalous to allow them to draw definite conclusions.

The original idea which prompted these experiments was that tissue cultured in a medium containing serum or plasma from the prospective recipient might become modified, perhaps by some alteration in the composition of the cell proteins, in such a way that it could better survive after transplantation. Lux *et al.* found however that, *in so far as culturing the tissue had any effect at all,* it made no difference whether the plasma used in the medium was obtained *from the prospective recipient, the donor,* or some other animal of the same species.

In the experiments described above considerable outgrowth of cells occurred in the cultures, and it was the outgrowing cells which were used for transplantation. More recently, however, Martinovitch (1950a) and Gaillard (1950) have advocated transplanting the original explant, after cultivation in a manner which allows little or no cell outgrowth. To achieve this both these workers recommend a fluid or semi-fluid medium containing only a minimal amount of embryo extract and, in addition, Martinovitch (1939, 1950a, 1951a) advises cultivation at 34°C. instead of 37°C. Both claim considerable success, Martinovitch with pituitary transplants in animals, Gaillard with parathyroid transplants in man.

Gaillard's findings have recently been reported in full (Gaillard, 1954). His patients were all suffering from parathyroid *tetany following thyroidectomy,* and eight out of twenty of the recipients under 32 years of age recovered following transplantation to the region of the axillary vessels of human foetal parathyroid which had been cultured *in vitro* for 14-21 days in a homologous medium which was changed at intervals by substituting more and more plasma and serum from the prospective recipient for foetal plasma and placental vein serum.

These reports should not be accepted uncritically. As Gaillard frankly admits he neither sought nor obtained histological proof that the transplants remained alive,

and it seems not unlikely that the patients recovered spontaneously. In the author's experience tetany following thyroidectomy is rare and when it occurs rarely persists for more than a few days; moreover, in clinics where persistent tetany is encountered more frequently, it is by no means unusual for spontaneous recovery to occur.

Preliminary Heterotopic Autotransplantation

Conway, Jerome, Stark and Joslin (1953) buried skin subcutaneously in mice for periods ranging from 2 to 25 days, and then transplanted it homologously to mice of an other strain, using the transparent chamber technique (p. 31). They found that thrombosis in the vessels of the bed rarely occurred with homografts pre-treated in this way, whereas it was usual with untreated grafts.

The survival time was extraordinarily variable, ranging from 4 to 38 days as judged by the gross appearance of the graft and the presence or absence of active circulation in the vessels of the bed. It is difficult to account for this variability, or to draw firm conclusions from such heterogeneous results.

Hormonal Stimulation of the Transplant in the Host

As we have seen (p. 56) there is evidence that transplants of certain endocrine tissues, including autotransplants in various sites and homotransplants in the anterior chamber of the eye, may survive if they are subjected to the appropriate hormonal stimulus but fail to do so in the absence of this stimulus. It is natural to ask therefore whether homotransplants of these tissues may be made to survive longer in sites where they would ordinarily be rapidly destroyed by administering very large doses of the appropriate hormone to the host.

Present indications are that the answer to this question is in the negative. Woodruff and Boswell (1953) found that adrenal homotransplants to the ovary in adrenalectomized rats, which were normally almost completely destroyed within 4 weeks, fared no better when ACTH was administered in a total dosage of 15 mg. per day. Similarly, Woodruff (1953b, 1954a) found that administration of thyrotrophin in a total dosage of 2 mg. per day failed to prolong the survival of subfascial thyroid homografts in thyroidectomized guinea pigs, and Krohn (1955b) found that injection of oestrogen, which stimulated the growth of autografts of vaginal epithelium transplanted to raw areas on the chest in rabbits, did not prolong the survival of homografts.

CREATION OF A BARRIER BETWEEN TRANSPLANT AND HOST

Local Use of Hyaluronic Acid

Conway, Stark and Joslin (1953b), working with mice, attached skin homografts to their beds with potassium hyaluronate, and to avoid or delay thrombosis in vessels near the grafts administered dicoumarol systemically. The presence of the potassium hyaluronate had however no apparent effect on the behaviour of the grafts.

This experiment was suggested by the hypothesis of Baesich and Wyburn (1947), to which reference has already been made (p. 52), that the survival of homotransplants of *cornea and cartilage* is facilitated by their high mucopolysaccharide content. Even if this is true, however, it seems very unlikely that mucopolysaccharides would confer protection on other kinds of homograft unless they could somehow be intimately incorporated in the tissue of the graft.

The Experiment of Hume and Egdahl

Hume and Egdahl (1955) enclosed kidney homotransplants in plastic envelopes with the object of determining whether isolation from the regional lymph nodes would modify the behaviour of transplants made by vascular anastomosis. They used 25 dogs as hosts and anastomosed the vessels by means of a specially designed prosthesis around which the neck of the plastic bag [illegible] be drawn tight. A watertight closure [illegible] achieved in [illegible] animals without impairing [illegible] blood supply to the transplant but there [illegible] no significant alteration in the period [illegible] which the transplant functioned or in the pattern of the histological reaction. An [illegible] feature of the technique was the [illegible] the zip fastener in closing the [illegible] so that the transplant could readily be [illegible] at intervals for inspection [illegible] biopsy.

[illegible] of interest to repeat these experiments [illegible] splenectomized hosts.

[illegible] Chamber Techniques

[illegible] membrane to isolate [illegible] cells of the host dates from [illegible] (1932) and his [illegible] collodion membrane they used [illegible] very [illegible]. In recent years much [illegible] membranes made of [illegible] nitrocellulose have become [illegible] have been used extensively [illegible] his colleagues (Algire, 19[illegible] Weaver and Prehn, 1954, 195[illegible] Weaver and Algire, 1954; Weaver [illegible] and Prehn, 1955), Woodruff (19[illegible]) and others.

Algire and his colleagues worked with mice. They used initially a transparent subcutaneous chamber modified by the addition of porous membrane between the transplant and the deep [illegible] of the skin of the host, but later they constructed special chambers (Fig. 35) and inserted them in the peritoneal cavity. In most experiments the

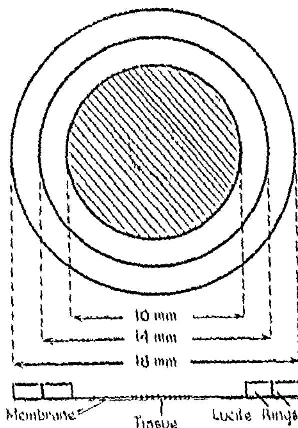


Fig. 35. Plan and elevation drawings to show the method of construction of Algire's diffusion chamber.

membrane was HA millipore*, having a thickness of 150 microns and an average pore size of 0.45 microns, but they also tried thinner membranes and membranes having larger pores which permitted the entry of some host cells. They used a variety of tissues including Harderian gland, embryonic lung and heart, and a mammary adenocarcinoma, and also a suspension of epidermal cells.

Woodruff studied split skin grafts in rats, separated from the panniculus carnosus muscle of the host by HA millipore by the technique illustrated in Figure 36. Dublie (1958) constructed very simple chambers consisting of a small square of membrane

*Manufactured by the Millipore Filter Corporation, Watertown, Mass. Porous membranes of various kinds can also be obtained from Carl Schleicher and Schuell Co., Keene, N.H., and the Wright Fleming Institute of Microbiology, St. Mary's Hospital, London.

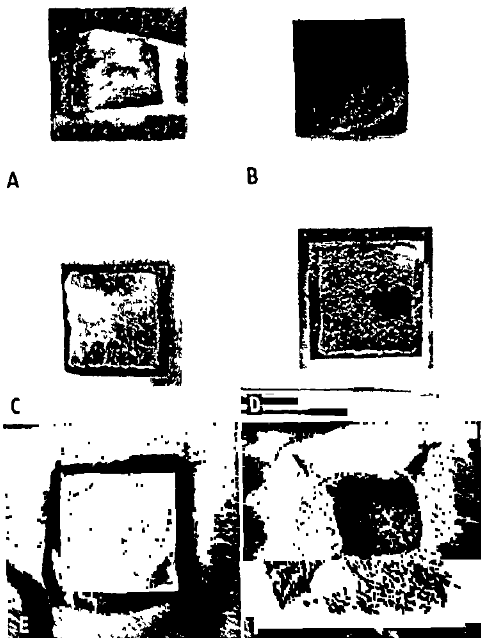


Fig. 36. Author's technique of grafting skin over porous membrane. A. Split skin graft (2 x 2 cm) placed raw surface upward on a sterile glass plate, and centred over a square of white paper (3 x 3 cm) beneath the plate. B. Square of membrane (2.5 x 2.5 cm) placed over the graft and centred by reference to the square of paper beneath the glass plate. C. Membrane with adherent graft inverted so that the outer surface of the graft is uppermost. D. Square of lightly greased sterile cellophane or adhesive cellulose tape, placed over graft and membrane, and made to adhere by gentle pressure with a glass roller. E. Sandwich of membrane, graft and cellophane placed on exposed panniculus carnosus muscle of host. F. Square of sterile plastic sponge placed over the sandwich and skin sutured across the corners. (Reproduced from *Annals of the New York Academy of Sciences* by courtesy of the publishers.)



[illegible] Section of homograft over Millipore membrane after 13 days. The surface epithelium has [illegible] but there is still viable epithelium in the hair follicles B. Part of the same graft after being [illegible] the donor for 7 days showing epithelial regeneration. Haematoxylin and eosin. ×150. [illegible] from *Annals of the New York Academy of Sciences* by courtesy of the publishers)

[illegible] piece of transparent adhesive [illegible]pe.*

[illegible] of these experiments have [illegible]ly consistent [illegible] shown in the first place [illegible] and Prehn, 1954; Woodruff, 1957) that homografts in diffusion chambers [illegible] effectively prevent the entry of host cells survive both in normal hosts and in hosts immunized by a previous non-protected graft from the same donor (or an animal of the donor strain) The nutrition of a solid graft† in a diffusion chamber [illegible] likely to be greatly impaired as compared with that of a vascularized graft and in Woodruff's experiments skin grafts looked decidedly under-privileged after 13-14 days on millipore membrane, but autografts, homografts in normal hosts, and homografts in immunized hosts all behaved similarly, and in each case irrefutable proof of viability was obtained by transplanting part of the graft back to the donor (Fig 37) These findings strongly support the view that host cells play a crucial part on the effector side of the homograft rejection mechanism. It might be argued that the membrane, despite the large pore size, somehow prevented the entry of humoral antibodies. It has been shown however (Algire, Weaver and Prehn, 1957) that normal plasma proteins and hetero-agglutinins readily pass through the membrane, and as we shall see (Chapter 8) heterografts, which are susceptible of destruction by humoral antibodies in the absence of host cells, fail to

*Many of the tapes on the market are unsuitable because they become soggy while the chamber is in the host, and may cease to adhere to the membrane. Two tapes (Nos 473 and 850) manufactured by the Minnesota Mining Corporation, Minneapolis, Minn., have however proved very satisfactory.

†As distinct from cells in suspension.

survive in diffusion chambers in immunized hosts.

Secondly, isologous transplants survive when enclosed in chambers containing also homologous spleen or lymph node cells from normal animals, but are destroyed in the presence of spleen or lymph node cells from immunized animals (Weaver, Algire and Prehn, 1957). This finding is consistent with the conclusion drawn from the experiments described in the preceding paragraph, but it is not in itself decisive because the homologous spleen and lymph node cells from immunized animals might conceivably have liberated significant amounts of humoral antibody within the chamber.

Thirdly, an unprotected homograft in an animal which has previously received a diffusion-chamber graft from the same donor, behaves like a first-set graft; in other words, homografts in diffusion chambers are not effectively antigenic (Woodruff, 1957c). This suggests that entry of host cells is necessary for the transport of antigen from a homograft, but it might be argued that the low rate of metabolism of a diffusion chamber graft is sufficient to account for the result.

Finally, there is some evidence to suggest that a homograft in a diffusion chamber in an immunized animal is modified in some way, as a result of which it is destroyed more quickly than it would otherwise have been when transferred to an unprotected site in the same host (Woodruff, 1957c). Once again experimental investigation is complicated by the impairment of nutrition imposed by the diffusion chamber technique, and the evidence is far from conclusive. As the author has pointed out, however, if this were confirmed it would suggest that humoral antibodies do play a part in the destruction of skin homografts, but that their role is equivalent to that of the opsonins of classical immunology.

It has been suggested that homografts of endocrine tissues in diffusion chambers might be used clinically for the treatment of myxoedema, parathyroid deficiency, Addison's disease and diabetes. There seems little prospect of this however because it has not so far been possible to maintain a sufficient amount of viable tissue even in laboratory animals to provide adequate replacement (Woodruff, unpublished; Egdahl, Roller and Varco, 1957).

CHAPTER 7

Specific Immunological Tolerance of Homotransplants

In this chapter* we shall discuss a group of phenomena which are as yet very imperfectly understood, and for which various descriptive terms have been introduced by different investigators. It would be premature to attempt to introduce a definitive terminology in our present state of knowledge, but in the meantime we shall use the term *specific immunological tolerance* to denote a state of reduced or absent reactivity to a particular antigen (or group of antigens) which would ordinarily evoke a specific reaction in consequence of previous exposure of the organism to the same, or a closely related antigen (or group of antigens). One way in which this condition may be produced is by exposing an animal to an antigen at a stage of development at which it is incapable of reacting in an immunologically mature way. Billingham, Brent and Medawar (1956a) have urged that the term *tolerance* should be restricted to tolerance produced in this way, but in the author's view it is preferable, as Snell (1954) has suggested, to adopt a wider definition such as the one given above. It is not claimed that all the instances of tolerance included in this definition are dependent on the same basic mechanism, but, on the other hand, it is not justifiable on the evidence available to dismiss this possibility.

We shall be concerned primarily with specific immunological tolerance of homografts,* but as we shall see in Chapter 8 tolerance can also be induced to heterotransplants and to various other types of antigen.

*This [illegible] is based on an article (Woodruff 1958) [illegible] Wochenschrift.

*Some investigators, including on occasion the present author, use the phrase *tolerance to* instead of *tolerance of*. It seems pedantic to object to this and the purist may perhaps derive some comfort from regarding *to* as an abbreviated form of *towards*.

INDUCTION OF TOLERANCE BEFORE THE DEVELOPMENT OF IMMUNOLOGICAL MATURITY

Burnet and Fenner (1948, 1949) drew attention to the fact that antibody forming cells react in two sharply different ways towards effete and damaged body constituents on the one hand and foreign organic matter on the other hand. To account for this they postulated that the expendable cells of the body possess a limited number of recognizable "self-marker" components, and in the light of the data then available concerning the immunological behaviour of immature animals they suggested further that the capacity to recognize self-pattern develops during embryonic or early post-natal life. They were thus led to predict that exposure to antigen at a sufficiently early stage of embryonic life would result in tolerance, and as we shall see this prediction has been confirmed in respect of some antigens.

The argument put forward by Burnet and Fenner would seem to apply with particular force to the antigens concerned in the immunity to homotransplants, and it is therefore not surprising that tolerance can be induced most easily in respect of these antigens. As far as other antigens are concerned much remains to be learned but it seems likely that the conditions under which tolerance can be induced differ not only in different species but for different antigens in the same species. In particular it seems likely that an animal does not become *immunologically mature* in respect of all antigens at the same time.

Erythrocyte Chimerism and the Discovery of Tolerance

Experimental embryology provides many instances of induction of tolerance to homologous tissue, the immunological significance of which was not appreciated at the time. Indeed, it is now apparent that the type of experiment in which tissues or organ rudiments are transplanted from one embryo to another would normally fail in birds, if the embryos behaved immunologically like adults, because of the reaction evoked by the transplants. Whether this is true also in amphibia remains to be seen because insufficient is known of the reaction of mature members of this class to homotransplants.

It is possible to find instances in other fields of biology in which immunological tolerance was produced experimentally without being recognized at the time, but the first clear demonstration of tolerance of the kind under consideration was provided by the work of Owen (1945) on the immunogenetic consequences of vascular anastomoses between cattle twins *in utero*. Cattle twins are commonly synchorial, and Owen showed by serological tests that most cattle twins at birth are erythrocyte chimeras, each calf having in its blood a mixture of its own erythrocytes and erythrocytes belonging to the cellular heritage of its twin. He attributed this state of affairs to exchange of blood during intra uterine life. It is now known that the state of chimerism persists for much longer than the life span of an erythrocyte and sometimes throughout the life of the animal, it follows, therefore, that erythropoietic cells must have been exchanged and have continued subsequently to produce erythrocytes of characteristic serological type (*see* Owen, Davis and Morgan, 1946, Stone, Stormont and Irwin, 1952). Erythrocyte chimerism appears to be rare in animals other than cattle, but has been demonstrated on three occasions in man (Dunsford, Bowley, Hutchison, Thompson, Sanger and Race, 1953, Booth, Plaut, James, Ikin, Moores, Sanger and Race, 1957, Nicholas, Jenkins and Marsh, 1957), in one pair of twin lambs (Stormont, Weir and Lane, 1953), and in twin chickens (Billingham *et al.*, 1956a).

Erythrocyte chimerism is but one manifestation of tolerance in dizygotic cattle twins resulting from admixture of placental circulations. This became apparent when Medawar and his colleagues (Anderson, Billingham, Lampkin and Medawar, 1951, Billingham, Lampkin, Medawar and Williams, 1952) showed that skin homotransplants interchanged between such calves would survive usually for long periods, and sometimes permanently, without regard to differences of sex or colour, whereas similar homografts between calves other than twins (including siblings of independent birth) were rapidly destroyed. More recently Neumann Sorensen, Gammeltoft and Simonsen (cited by Simonsen, 1955) have shown that a whole kidney transplanted by vascular anastomosis from one calf twin to the other of a pair showing erythrocyte chimerism functioned for five months.

One would expect that twin chimeras when they occur in other species would be

tolerant of each other's tissues. This has been confirmed in a pair of human twins of opposite sex showing blood chimerism by Woodruff and Lennox (1959), who interchanged small full-thickness skin grafts between them and observed during the course of the next 12 months that the grafts retained their original size and showed no microscopic evidence of breakdown (Fig. [illegible]). This is in sharp contrast with the behaviour of skin grafts interchanged between normal human dizygotic twins which have been found to survive for only 3-4 weeks (Bishop, 1955, Rogers and Allen, 1955a, b, Sniderman, Rogers and Allen 1956). Histological studies in the chimeras reported by Woodruff and Lennox (*op. cit.*), including chromatin sexing (Barr and Bertram, 1949; *see also* Barr, 1956, Lennox, 1956), suggest however that in the male twin the cells of the graft have nearly all been replaced by host cells, though the state of chimerism has persisted.

Induction of Tolerance to Homotransplants in Animals

[illegible] been discussing immunolog[illegible] produced more or less in[illegible] arising during the course of [illegible]pment. Once the significance [illegible]mena was appreciated the [illegible] Burnet and Fenner (1948, 1949) sugg[illegible] to try deliberately to induce toler[illegible]perimentally, and during the next few [illegible] several investigators began to work [illegible] problem.

Billingham, Brent and Medawar (1953, 1955, 195[illegible]) injected mouse embryos of the CBA stra[illegible] *utero* on the 15th or 16th day of pregnancy with 0.01 ml. of a suspension of cells prepared by chopping up testis, kidney and spleen from a mouse of strain A. The injection was given with a fine needle inserted through the abdominal wall after an incision had been made in the skin, and by this means it was found possible to inject most, though not always all, of the embryos. The offspring which survived received skin homotransplants from CBA donors when they were eight weeks old, and in most of them the transplants survived longer than normal, indicating that tolerance had been induced. The degree of tolerance varied from animal to animal, some transplants surviving only a few days longer than normal, others surviving indefinitely. A few animals showed no evidence of tolerance and it was concluded that these had been inadvertently missed when their litter mates were injected.* Mice injected at birth did not become tolerant nor, on the other hand, did they develop increased resistance to skin homotransplants from the donor strain.

It will be observed that the inoculum contained no cells derived from skin, and it was shown further than it need not contain epithelial cells of any type. The tolerance always proved, however, to be immunologically specific, CBA mice made tolerant of A strain tissues always reacting normally against skin from a donor belonging to a third strain AU.

Billingham *et al.* (1953) also experimented with chicks. They found that it was possible to transfuse 0.2 ml. whole blood from an 11-12 day Rhode Island Red embryo into a chorio-allantoic vein of a White Leghorn embryo of the same age. By transplanting skin from the original donor to the original recipient 14 days after hatching it was shown that this procedure had conferred a high degree of tolerance.† In other experiments newly hatched Rhode Island Red donors were killed, portions of various tissues were transplanted to the chorio-allan-

*There is no reason to doubt this explanation but all uncertainty can be avoided by adding a little trypan blue to the cell suspension, as described by Koprowski (1955). Injected and non-injected animals can then be readily distinguished at birth by their colour.

†In mice the availability of closely inbred strains makes it possible to use any member of the donor strain instead of the original donor, but at present this is not often possible with other species since genetically homogeneous strains are seldom available.

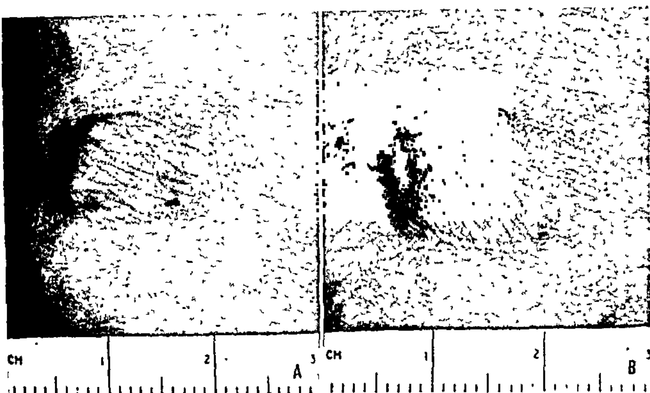


Fig. 38. Skin homografts exchanged 6 months previously between dizygotic human twins showing blood chimerism. A. Graft on female twin. Note the coarse ridge pattern of the graft and the fine pattern of the surrounding host skin. B. Graft on male twin. Here the ridge pattern of the graft is fine and that of the surrounding skin is coarse. (Reproduced from the *Lancet* by courtesy of the publishers.)

toic membranes of 10-11 day White Leghorn embryos, and portions of skin were stored* and used later as test transplants. It was found that transplantation to the chorio-allantoic membrane conferred tolerance less regularly and less completely than injection of whole blood. Later, Billingham *et al.* (1956a) found that intravenous injection of whole blood to newly hatched chicks conferred partial tolerance to skin from the blood donor.

Hašek (1953a, b) devised an ingenious technique for producing parabiosis of avian embryos through the chorio allantoic membrane, and showed that chicks which had been united in this way from about the 10th day of embryonic life until hatching failed to produce antibodies when challenged later with each other's blood, and (Hašek, 1954) would readily accept homotransplants of each other's skin, whereas normal chicks produce haemagglutinins when injected with homologous erythrocytes and reject homotransplants of skin after a few days. Experiments performed by Hašek and Hraba (1955b), in which immune serum was prepared in a third chicken by injecting erythrocytes from one of a pair of birds which had formerly been joined in parabiosis, and absorbed against erythrocytes from the other member of the pair, revealed no evidence of erythrocyte chimerism. Further investigations by Billingham *et al.* (1956a), using more refined serological tests, have shown that this observation was erroneous and that erythrocyte chimerism does occur in birds subjected to embryonic parabiosis. It quite often happens, however, that tolerance resulting from the procedure is incomplete, as shown by the fact that a homotransplant of skin from the former partner, though accepted for a considerable time, is eventually destroyed. In this event

* At −[illegible] C. after being treated with glycerol

the chimerism gradually disappears, but even when it has disappeared completely some degree of tolerance to the former partner's erythrocytes may still be demonstrable.

In other experiments Hašek and his colleagues found that injection of washed homologous erythrocytes intravenously or subcutaneously to newly hatched chicks rendered some of them incapable of forming agglutinins when challenged 8-18 weeks later (Hašek, Hraba and Esslová, 1956), but did not produce tolerance to donor skin (Hašek, Lengerová and Maternová, 1955).

Ripley (1953), working in Owen's laboratory, succeeded in producing erythrocyte chimerism in rats by injecting foetal rats intravenously with homologous haemopoietic cells.

The author, after numerous unsuccessful attempts to induce tolerance in rats by transplanting homologous skin to rat embryos *in utero,* abandoned this approach and began to study the effect of injecting suspensions of homologous spleen cells to



Fig. 39 Skin homografts in an albino rat made specifically tolerant by injection of spleen cells at birth. A. First graft after 12 weeks. B. Second graft after 4 weeks. The donor was a black and white hooded rat and part only of each graft was black.

both foetal and new born rats using black and white hooded rats as donors and Wistars as recipients * It was found (Woodruff and Simpson 1954 1955b) that subcutaneous injection on the day of birth regularly conferred a high degree of tolerance to homotransplants of skin from the same donor (Fig 39) while injection four weeks after birth resulted in the development of immunity Subcutaneous injection two weeks after birth conferred a high degree of tolerance in a few recipients and a lesser degree of tolerance in others but as a rule caused neither tolerance nor immunity

Treatment with cortisone for 12 days immediately following the injection of homologous cells did not decisively modify the effect of the injection (Woodruff and Simpson 1955b) on the other hand injection of homologous cells at the age of two weeks to rats which had received a daily injection of cortisone in large dosage from birth regularly conferred a high degree of tolerance (Woodruff 1957a) It was therefore concluded that treatment with cortisone delays the development of immunological maturity in rats

These experiments showed for the first time that a high degree of tolerance to homotransplants could be induced in a mammal by post natal injection of donor cells They thus established the importance of the rat as an experimental animal for work in this field and encouraged attempts to induce tolerance after birth in other species including man (*v. infra*) They also represent the first successful attempt to retard immunological maturation by experimental means

In later experiments Woodruff and Sparrow (1957) investigated the effect of injecting spleen cells intraperitoneally on the day of birth and 3 6 9 and 12 days thereafter The amount of suspension injected was 0.2 ml per g body weight corresponding to a mean dosage of 18 26 42 60 and 67 million cells for animals aged 0 3 6 9 and 12 days respectively

Thirty nine out of 94 injected animals died before they were due for skin grafting Some of these failed to thrive almost from the time of injection and died 2-4 weeks later after a precarious existence during which their rate of growth was far below normal the others developed apparently normally for 4-6 weeks and then died suddenly usually with symptoms of enteritis The significance of these deaths will be *considered shortly* (p 125)

The animals which survived received a skin homograft at the age of 8 weeks from the cell donor and in most of them—including 10 out of 11 animals injected at the age of 12 days—the grafts survived without change of size for the 12 weeks during which they were kept under observation and at the end of this time were histologically indistinguishable from autografts

While these experiments were in progress Billingham and Brent (1957a) were studying the effect of intravenous and intraperitoneal injection of spleen cells to new born mice Initially they used CBA donors and A strain recipients Nearly all the mice injected with 1-10 million cells within 24 hours of birth and tested at the age of 8 weeks with homografts of donor strain skin were found to be highly tolerant After 24 hours the chance of producing a high degree of tolerance declined reaching a level of about 50 per cent at the age of 4 days and animals injected after 7 days sometimes showed evidence of weak immunity when they were subsequently skin grafted Eleven out of 64 of the injected animals died before they were due to be grafted and nearly 50 per cent of the fully tolerant mice showed extensive or complete involution of their axillary brachial and inguinal lymph nodes

Intraperitoneal injection also resulted in tolerance in a fair proportion of animals though it did not appear to be as effective

* The strains of rats used were not genetically homogeneous so that tests of tolerance to transplants from the original cell donor were always used

as intravenous injection. Subcutaneous injection however, was ineffective.

Observations on the effect of intravenous injection with other strain combinations revealed considerable variation both in the incidence of tolerance and in mortality. C57 mice injected with A strain cells for example never became highly tolerant, and [illegible] mice injected with either C57 or [illegible] cells invariably died.

Billingham and Brent found that cell suspensions prepared from the lymph nodes or spleen of tolerant mice were capable of immunizing normal animals of the same (host) strain against homografts of donor strain tissue. They concluded therefore that the cells used to produce tolerance, or their mitotic descendants, had become established in these organs.

Puza and Gombos (1958a, b, and personal communication) succeeded in producing a high degree of lasting tolerance to skin homografts by exchange transfusion in puppies 3-12 days after birth. The same procedure in animals aged 51 days or more did not produce tolerance, but it is not clear from the published reports whether it produced any degree of immunity.

As [illegible] Stein, Peterson, Grazer and Longmire ([illegible]) have succeeded in producing a high degree of tolerance to homologous skin in rats by intracardiac injection of 0.05 ml whole blood at birth. The donors were rats of the Long Evans strain, and the blood of several donors was pooled. The recipients were Wistars. They were skin grafted at the age of 3 weeks from one of the pool donors and 18 out of 46 grafts were still surviving after [illegible] days or longer, whereas control homografts to untreated hosts were destroyed within 11 days. It seems likely that even more striking results might have been obtained if blood from a single prospective donor had been used instead of pooled blood.

Various attempts—some of them successful—have been made to induce tolerance to homologous tissue by using solid transplants instead of injecting cell suspensions. As mentioned earlier, the author's attempts to do this in rat embryos failed for technical reasons. Schinkel and Ferguson (1953) devised a satisfactory technique for transplanting skin to sheep embryos, but found that homotransplants to embryos of 100 days' gestation evoked a marked reaction and were destroyed. In chicks however Cannon and his colleagues (Cannon and Longmire, 1952; Cannon, Weber and Longmire, 1954) showed that skin homotransplants made the day after hatching survived on average much longer than those made to chicks aged seven days and older, and sometimes permanently; and Hraba and Hašek (1956) found that skin homografts to newly hatched ducks survived permanently in about 65 per cent of cases. These observations have been taken to indicate that skin transplants to sufficiently young birds induce a state of tolerance which facilitates their own survival. Cannon (1957), however, has questioned this interpretation on two grounds. In the first place he has drawn attention to the fact that in his experience skin grafts to newly hatched chicks were sometimes rejected as late as two months after grafting, but if they survived three months then they continued to survive permanently. Secondly, he found that not more than 50 per cent of successful grafts, when grown to adult age, could be returned to the donor without evoking a reaction. He has suggested therefore that, while the initial survival of the graft is due to the host's inability to react in an adult way, the graft continues to survive because it gradually assumes the tissue specificity of the host and not because the host becomes tolerant. If this explanation is correct then clearly here is yet another situation in which the author's hypothesis of the critical period (p. 79) holds good.

This type of experiment has been carried a stage further in rats by Medawar and

Woodruff (1958), who showed that Wistars which received at birth a small transplant of skin from an adult hooded rat were subsequently partially tolerant, and sometimes highly tolerant, to a further massive skin transplant from the same donor

It is apparent from the experiments which have been cited that the consequences of exposing an immature animal to homologous cells depends among other things on the species of animal used, the genetic constitution of donor and host, the type and number of cells used, and the route by which the cells are introduced into the host

Another factor which may be of importance is the length of time during which the antigen can act during the adaptive period Hašková (1957) has emphasized this and has shown in birds that repeated injections may be much more effective than a single injection.

The Reaction of Graft Against Host

As we have seen intraperitoneal or intravenous injection of homologous spleen cells in new born mice (Billingham and Brent, 1957b) or rats (Woodruff and Sparrow, 1957, Woodruff, 1957b, d) may result in a peculiar and often fatal syndrome to which Billingham has given the name *runt disease*, the main features of which are wasting (Fig 10), hypoplasia of lymphoid tissue and liability to intercurrent infection Moreover, as Simonsen (1957) has shown, intravenous injection of homologous spleen cells, or leucocytes from the buffy coat layer of peripheral blood, to chick embryos three days prior to hatching, results in severe haemolytic anaemia associated with splenomegaly, and replacement of myelopoietic and erythropoietic cells in the spleen, bone marrow and thymus by proliferating, pyronino-



Fig 10 Two CBA mice aged 27 days from the same litter The smaller, which weighed 2 7 g and showed typical signs of runt disease, received at birth an intravenous injection of 4 million homologous nucleated spleen cells from an adult C57 black mouse The larger which weighed 8 8 g and appeared normal for its age received at birth an intravenous injection of 4 million isologous nucleated spleen cells.

philic reticulo-endothelial cells, and as a rule death in the first or second week after hatching

There is good evidence that these sequelae are manifestations of an immunological reaction by the transplanted cells or their descendants against the host

In the first place, as we have seen, the organs overtly affected are those in which the injected cells have been shown to settle out electively and establish themselves (Billingham and Brent, 1957b).

Secondly, injection of isologous cells, and also of homologous cells from a sufficiently immature donor, appears to be quite innocuous (Simonsen, 1957)

Thirdly, according to Billingham and Brent, when tolerance is induced by cells which are not immunologically reactive, the tolerant animal shows no pathological changes *

It would help to clinch the matter if it could be shown that immunologically competent cells from an animal of one strain (X) which had previously been made tolerant of cells of members of a second strain (Y) were innocuous when injected into immature members of the Y strain Experiments of this kind are in progress in the author's laboratory.

A curious fact, which may prove to be of therapeutic importance, is that subcutaneous injection of homologous spleen cells, which in rats may result in a high degree of tolerance, has so far not been found to cause runt disease (Woodruff and Sparrow, 1957)

Tolerance and the Immunogenetics of Transplantation

If it is true that every normal nucleated cell in an individual (with the exception of germ cells which have undergone meiosis) carries the same set of T antigens (p. 93), then the tolerance induced by normal cells of one type should extend to all other normal cells from the same donor. The available evidence is entirely consistent with this hypothesis. Dizygotic calf twins in which there has been an exchange of haemopoietic cells resulting in erythrocyte chimerism, as already mentioned, have been shown to be tolerant of each other's skin, and on one occasion tolerance to a transplanted kidney has been demonstrated. Tolerance induced experimentally in various species by injection of spleen cells has been shown to extend to skin, and in addition in the rat has been shown to extend to homotransplants of adrenal cortex (Woodruff and Sparrow, 1958, Medawar and Russell, 1958) and thyroid (Woodruff and Sparrow, 1958). On the other hand, mammalian erythrocytes, which do not carry the T antigens, and nucleated cells subjected to freeze-drying and other procedures which destroy these antigens, cannot induce tolerance (Billingham *et al*, 1956a) It would be of great interest to determine whether tolerance could be induced with antigenic material from prospective donor cells, but so far this has not yet been achieved

If cells of an animal A, when injected into another animal B, produce a state of immunity to the cells of a third animal C, then we may conclude that A and C have some antigens in common. If, however, A's cells when injected into B produce a state of complete tolerance to C's cells then we may draw the more far-reaching conclusion that all of C's antigens are represented in A. Thus, for example, if two homozygous strains, each of which rejects transplants from the other, are crossed, the F1 progeny should possess all the antigens of both parent strains, and, if the theory outlined is correct, cells from the F1 progeny should produce tolerance to transplants from both parent strains when injected into a suffi-

*There is no reason to doubt the truth of this statement, but care is needed to avoid the fallacy of assuming what one is trying to prove It seems best to base the hypothesis of a graft against host reaction on the other reasons given above, and to use the capacity of cells to cause reactions of this kind as a criterion of their immunological competence.

ciently immature member of a third strain *but cells of either parent strain alone should* not induce tolerance to transplants from F1 progeny

This appears to hold good for transplants of normal tissues

With neoplastic cells the situation is more complex and the experimental data are meagre Billingham *et al* (1956a) found *that 37 per cent of new born CBA mice* injected subcutaneously with cells and cell clumps from a transplantable carcinoma which grew normally in A strain mice but not in CBA mice developed a tumour and some but not all of these recipients later showed tolerance of varying degree to A strain skin Subcutaneous injection is however *not an effective method of producing* tolerance with normal cells in new born mice and there seems no reason why it should prove more effective with neoplastic cells so that a high degree of tolerance could scarcely have been expected Koprowski (1955) found that an ascites tumour which normally grew in C3H mice but not in Swiss *mice could be successfully transplanted to* 16 17 day embryo Swiss mice of the ICR strain and also to adult ICR mice which had received during embryonic life an injection of whole blood from an adult C3H or F1 (C3H × DBA) mouse He made the further interesting observation that the antigenic character of the tumour altered on passage *through a host made tolerant by* one or other of the procedures just described and attributed this to a process of selection operating on a population of antigenically diverse cells

These experiments show that at least some degree of tolerance to both neoplastic and normal cells may be induced with neoplastic cells * and conversely *that tolerance* to neoplastic cells may be induced with normal cells They leave open however the question of whether normal and neoplastic cells differ in their antigenic constitution—as they would be expected to do if the somatic mutation theory of cancer is correct—though Koprowski's last mentioned observation points in this direction To clinch the matter it would suffice to show either *that complete tolerance to normal cells can* not be induced with neoplastic cells or *vice versa* The former could be tested experimentally quite easily but the latter is more difficult owing to the fact that tumour transplants may progress and even kill the host in the face of resistance sufficient to destroy a transplant of normal tissue and in consequence do not provide a sensitive index of complete tolerance

* Billingham *et al* (1956a) took steps to ensure that the transplanted carcinoma contained relatively few stromal cells.

Partial Tolerance to Homotransplants

*It has already been pointed out that toler*ance is not an all or nothing phenomenon but may occur in every degree from very slight to complete tolerance This is well illustrated by the behaviour of homotransplants of skin In a completely tolerant animal a homotransplant from the appropriate donor behaves just like an autotransplant and subsequent homotransplants from the same donor all behave similarly In a host which is highly but not completely tolerant a homotransplant sooner or later begins to contract in size and to show areas of scabbing and is eventually destroyed A very slight degree of tolerance can be recognized only if the period of graft survival in the absence of tolerance can be predicted accurately and is indicated by survival slightly beyond the expected time (*see* e g Woodruff and Simpson 1955*b* Billingham *et al*, 1956a)

The behaviour of second transplants in partially tolerant hosts is of great interest

Billingham *et al* (1956a) observed the behaviour of second skin transplants from the same donor in 16 mice which had shown partial tolerance towards the first transplants but had eventually rejected them

These second transplants survived on average for about the same time as normal first transplants in hosts which were neither immune nor tolerant. Two mice received third transplants, and these survived slightly longer than normal second transplants. Billingham *et al.* (1956a) concluded that "the state of tolerance represents a permanent impairment of the faculty of immunological response," but it would seem unwise to accept this conclusion on the rather meagre evidence presented, especially in view of its important theoretical implications. Apart from the two animals which received third transplants the findings indicate only that rejection of a homotransplant in a partially tolerant host does not necessarily leave the animal in a state of immunity. To prove conclusively that tolerance is not abolished by breakdown of a transplant it would, in the author's opinion, be necessary to show that a second transplant survived longer than a first transplant survives in a normal host.

Another finding with important theoretical implications is that, in a host which is highly but not completely tolerant, a first transplant which has been established for a considerable time may continue to survive without any change in its gross appearance while a second transplant from the same donor is destroyed. This was observed by Weber, Cannon and Longmire (1954) in chickens and by Woodruff and Simpson (1955b) in rats, and provides further evidence for the hypothesis of the critical period (p. 79). The mechanism of this phenomenon has not been elucidated but it may be due, at any rate in part, to complete replacement of the vascular endothelium of the transplant by endothelium of host origin (*see* Billingham *et al.*, 1956a).

It must be emphasized that the rejection of an orthotopic skin homograft in a partially tolerant host may be a very long drawn out process, and in an extreme case the graft may be replaced by host tissue with little or no gross evidence of breakdown. This process can be distinguished from true survival because the structure of the skin is not fully restored, but the observation of Breedis (1954) that hair follicles and sebaceous glands can regenerate from the epithelium of scars in rabbits indicates the need for very great care in interpreting the histological findings. Probably the best safeguard is frequent inspection of the grafts, and very thorough histological examination of those showing any degree of contraction.

Maternally Induced Tolerance

It is natural to ask whether tolerance to maternal tissue occurs as a result of accidental passage of cells from mother to foetus.

It was suggested independently by Brambell (cited by Booth, Dunsford, Grant and Murray, 1953), Mitchison (cited by Booth *et al.*, 1953) and Owen (*see* Owen, Wood, Foord, Sturgeon and Baldwin, 1954; Owen, 1956) that if, in human beings, Rh antigens were to enter an Rh-negative foetus, they might lower or even abolish its power to react against Rh antigens in later life. If the foetus in question were a female, and gave birth when she grew up to a male Rh-positive child, the risk of this child suffering from haemolytic disease of the new-born would be correspondingly reduced or abolished. In the light of these considerations Booth *et al.* (1953) investigated the Rh grouping of the maternal grandmothers of first-born Rh-positive infants having Rh-negative mothers, but found that incidence of haemolytic disease in these infants was unrelated to the Rh group of the grandmother. Owen *et al.* (1954), on the other hand, showed by serological tests that sensitization to Rh antigens occurred less frequently in Rh-negative women whose mothers were Rh-positive than in those whose mothers were Rh-negative; curiously enough, however, they found no corresponding difference in the incidence of haemoly-

tic disease in Rh-positive infants of the next generation, and *in this respect* confirmed *the findings* of Booth *et al.*

Billingham *et al.* (1956a) sought for evidence of tolerance to maternal skin in mice and rabbits but failed to find it. In guinea *pigs* however, which have a relatively long *gestation period, they observed* very prolonged survival of skin homotransplants from mother to offspring in 2 out of 49 recipients, and inclined to the belief that this was due to the transfer of maternal cells *during intra-uterine life.*

Peer (1956) reported long survival of a transplant of maternal skin in a human infant, but the significance of this observation is obscured by the fact that the infant *had* received an *injection of blood from* the mother when it was three weeks old. Later (Peer, 1957; Peer, Bernhard and Walker, 1958; Peer and Walker, 1958), he reported that maternal skin transplanted to infants or older children sometimes survived for long periods (more than a year in some cases) whether the infant had been injected with maternal blood during the neonatal period or not, whereas paternal skin did not survive longer than 20 days.* He made the further surprising observation that infant's skin transplanted to the mother also sometimes survived throughout the period of observation (2 years in one case).

The findings suggest that maternal induc*tion of tolerance does sometimes* occur but is, in most species at any rate, a rare phenomenon. This is not surprising because it seems likely that if transfer of cells occurs at all it will take place in both directions, and in this event the survival of the foetus might well be prejudiced in consequence of immunity to foetal tissues developing in the mother.

*Kearns and Reid (1919) had previously reported a case in which skin homografts from both mother and father behaved like autografts, but this may conceivably be explained by the fact that the child (a boy age 9) had previously received blood transfusions from both parents.

Can Tolerance to *Homotransplants* Be Induced in Man?

In an attempt to find out whether tolerance to homotransplants could be induced in man, the author (Woodruff, 1957b) injected two new-born babies intramuscularly with approximately 300 million leucocytes from their respective fathers, and six *months* later challenged each infant with a small transplant of its father's skin. The transplants took well and behaved exactly like autotransplants for four weeks, but thereafter *they* began to contract, *apparently* owing to the formation of scar tissue. It is difficult to assess the significance of these findings in the absence of information about the behaviour of skin transplants from *fathers to untreated infants,* but the appearances suggest that, while complete tolerance *had not been achieved, a state of partial* tolerance had been induced.

More complete tolerance might have been obtained if the leucocytes had been injected intravenously but this was deemed inadvisable because of the danger of a graft against host reaction.

If complete tolerance to homotransplants could be induced in human beings by a technique which was both simple and safe there would be much to be said for carrying out such a procedure routinely in early infancy, using one or more adults as donors. If then the child later sustained an extensive deep burn, skin transplants *from the same* donor or donors could be used to provide permanent replacement of the lost skin. It would probably be convenient to use the father as a donor though there is no compelling biological reason for this.

The Abolition of Tolerance

It has been shown by Billingham *et al.* (1956a) that tolerance to a skin homotrans*plant can be permanently abolished, and* the transplant caused to break down, by injecting the tolerant animal with lymph

node cells from a non-tolerant animal isogenic with it. This holds good even if the animal from which the lymph nodes are obtained has had no previous treatment, but tolerance can be abolished much more rapidly by using the regional lymph nodes from an animal actively immunized against donor strain skin. Thus, for example, Billingham *et al.* found that a homotransplant of skin from a CBA mouse in a tolerant A strain host showed evidence of a reaction 14-40 days after injection of normal A strain lymph node cells and was completely destroyed within 19-80 days, whereas after injection of cells obtained from the regional lymph nodes of an A strain mouse actively immunized against CBA skin the tolerated graft showed evidence of a reaction after 3 or 4 days and was destroyed within 6-15 days. Injection of lymph node cells from a mouse of a third strain AU which had been actively immunized against CBA skin caused at most a slight and temporary reaction in the tolerated graft, and Billingham *et al.* therefore concluded that the abolition of tolerance by injection of cells from an animal which is not tolerant is contingent on the survival of the injected cells.

This phenomenon is not confined to skin grafts for it has been shown by Medawar and Russell (1958) that adrenal homografts surviving in tolerant mice can be destroyed in the same way.

Nature of the Phenomenon

The observations discussed in the preceding section would seem to imply, as Billingham *et al.* (1956a) have suggested, that tolerance is due to a central failure of the mechanism of immunological response, and this accords well with the hypothesis of Burnet and Fenner (p. 118).

There has been much discussion as to whether antibody may continue to be produced in the absence of antigen (*see* e.g. Burnet and Fenner, 1949), and it is pertinent to ask whether the persistence of a state of tolerance is contingent on the persistence of the antigen by which the tolerance was induced. In an attempt to answer this question Medawar and Woodruff (1958) performed an experiment in which new-born Wistar rats each received a small homotransplant of skin from a young adult hooded donor. Four weeks later the transplants in about half of the recipients were excised together with the underlying subcutaneous tissue and a wide margin of surrounding host skin. After a further four weeks all the animals bearing transplants, or from which transplants had been excised, received a second and larger transplant to the opposite side of the body from the original donor. In both groups these second transplants survived on average longer than homotransplants in normal animals, but except in a few instances were eventually destroyed. It would be premature to try to draw firm general conclusions until further data are available, but it seems clear, firstly, that the initial transplant induced a state of at least partial tolerance, and secondly, that this tolerance was not immediately abolished when the transplant was removed.

This experiment throws some light on another question. It seems reasonable to suppose that the antigenic stimulus of the first grafts impinged mainly on the regional lymph nodes, yet the tolerance which resulted was clearly not merely regional since it extended to the opposite side of the body. In the author's opinion the most likely explanation is that there is a constant coming and going of lymphoid cells in the regional nodes and that these cells are specifically modified during their period of residence. Alternatively, cells which have been specifically modified by contact with antigen might somehow induce a similar modification in their fellows.

It should be possible to discriminate between these hypotheses by seeking to deter-

mine whether tolerance can be conferred by a process of adoptive transfer, but so far no one seems to have thought it worth while to try this

Both hypotheses assume that there are such things as specifically tolerant cells as well as tolerant animals There seems no reason to doubt this but the current experiments of the author designed to determine whether or not immunologically reactive cells from tolerant hosts are able to cause runt disease in new born animals of the donor strain (p 125) should provide a decisive answer

Assuming that cells can be made tolerant what is the nature of the modification which they undergo?

If we accept the view of Burnet (1956) that antibody formation is akin to the formation of adaptive enzymes by bacteria it seems a reasonable guess that a cell which encounters an antigen before it is capable of elaborating a specific antibody might form a precursor of some kind as a sort of abortive step in the adaptive process, and such a cell and its descendants might conceivably continue to elaborate this ineffective precursor whenever they encountered the same antigen Alternatively, if we accept Burnet's (1959) more recent clonal selection theory of acquired immunity, tolerance of a particular antigen implies that all cells capable of reacting against it were destroyed when the antigen was first introduced into the host, and that more cells with similar reactivity have either not appeared or have in their turn been eliminated

INDUCTION OF TOLERANCE AFTER THE DEVELOPMENT OF IMMUNOLOGICAL MATURITY

The phenomena now to be considered form a very heterogeneous group and in the absence of a unifying hypothesis linking them together it is necessary to describe each one separately For the same reason it seems desirable, wherever possible, to adopt an eponymous nomenclature.

Enhancement

Experiments have been reported by several groups of investigators in which the survival of tumour homotransplants, and to a less extent homotransplants of normal tissue, has been facilitated by prior injection or transplantation of tissue of the same kind which has been killed by heating, freezing and thawing, or freeze-drying, or by injection of tissue extracts The phenomena have been given various names such as "the XYZ effect" (Casey) and "the enhancing effect" (Casey, Snell) It seems desirable to use a single term and the one used here is *enhancement* This is admittedly often inappropriate, especially in relation to the behaviour of transplants of normal tissue, but has been adopted on the ground of familiarity

Observations with Tumour Transplants

Flexner and Jobling (1907a, b) found that the growth of tumour transplants in rats was enhanced by prior injections of heat killed tumour tissue.

Casey and his associates (Casey, 1932, 1933a, b, 1934a, b, 1939, 1941, Casey, Meyers and Drysdale, 1948) obtained similar results with tumour homotransplants in mice and rabbits, using prior injections of material obtained by freezing the corresponding tumour until it was no longer viable. The enhancing effect was long lasting, and in some experiments in rabbits was shown to be still present after seven months Injection of material obtained by freezing a different homologous tumour was also effec-

tive sometimes, though never to the same extent as material from the tumour subsequently to be transplanted. Heterologous material has been shown to be quite ineffective (Kaliss, 1952b; and others).

Snell and Kaliss, and their associates, found that transplantable mouse tumours could be made to grow progressively in resistant strains by pretreating the host with large doses of lyophilized tumour tissue (Snell, Cloudman, Failor and Douglass, 1946; Kaliss, Borges and Day, 1954); with supernatant fluid obtained by centrifuging saline homogenates of the tumour in question (Kaliss, 1955a), with large doses of lyophilized normal spleen, kidney or liver from the donor strain (Kaliss and Snell, 1951; Kaliss, 1952a, Snell, 1952b; Day, Kaliss, Aronson, Bryant, Friendly, Gabrielson and Smith, 1954), and, in some instances, with fresh homogenates of normal tissue (Kaliss, Jonas and Avnet, 1950). Enhancement can also be produced by lyophilized tissue from a different strain provided that this third strain and the tumour donor strain have in common an H-2 factor (p. 62) which is lacking in the host (Snell, 1954), and it would seem therefore that the enhancing substance in mice must be a product of the H-2 locus.

In the experiments cited the effective total dose of lyophilized tumour tissue was 30-50 mg. and according to Kaliss and Newton (1949) a small dose (0.05 mg.) resulted in increased resistance to subsequent tumour transplants. The usual interval between the last injection and the transplantation of living tumour was between one and three weeks, but in later experiments (Kaliss and Day, 1954) the enhancing effect was shown to persist for as long as 46 weeks after the injection; injection of lyophilized material after transplantation of a tumour, however, was ineffective.

Kaliss and Molomut (1952) demonstrated a remarkable phenomenon which Snell (1952) described as "something equivalent to a passive transfer of the enhancing effect." They injected C57 black mice, and also rabbits, with antigens prepared from mixed normal and tumour tissues from strain A mice. Serum from these animals was later injected into normal C57 mice, which then became susceptible to a transplantable strain A tumour to which they were normally almost completely resistant. Further work (Kaliss, Molomut, Harriss and Gault, 1953; Kaliss, 1955a; Kaliss and Kandutsch, 1956; Kaliss, 1956a, 1957) points to the conclusion that this phenomenon is dependent on the presence of antibodies in the serum. In the first place it has been shown that the activity of the serum is associated with the globulin fractions, and probably specifically with the gamma globulin. Secondly, the effectiveness of the serum depends on the interval which elapses between injecting antigen and bleeding the animal, serum obtained after an interval of three weeks being maximally effective, but serum obtained after eight months still showing a significant effect. Thirdly, the effect of an injection of active serum prior to tumour transplantation falls off rapidly during the first seven days, but, on the other hand, injection up to as long as 10 days after tumour transplantation has a significant effect.

Kaliss (1955a) showed also that the enhancing effect of an injection of supernatant fluid from a tumour homogenate is abolished, except when the dose of supernatant fluid is very large, by simultaneous injection of cortisone, and (Kaliss, 1957) that the effect is restored if antiserum, prepared by immunizing mice of the host strain with saline homogenates of either normal donor strain spleen or lyophilized tumour tissues, is injected in addition.

Mitchison and Dube (1955) showed that enhancement could be abolished in mice by injecting cells from the regional lymph nodes of an actively immunized mouse of the host strain, and in this respect enhancement resembles tolerance produced by ex-

posure to antigen during immunological immaturity On the other hand injection of lymph node cells from an untreated member of the host strain, which also abolished tolerance produced by exposure during immunological immaturity, gave indecisive results with enhanced mice

Another phenomenon, which appears at first sight to resemble Mitchison's but is almost certainly quite different in nature, has been described by Snell (1956), who used an ascites variant of the A strain sarcoma used by Kaliss, which grew progressively in about 50 per cent of untreated C3H mice subjected to enhancement, and studied the effect of inoculating a mixture of tumour tissue and minced lymph nodes from normal A or C3H mice He found that when donor strain (A) nodes were used growth of the tumour in enhanced hosts was restricted, whereas when host strain (C3H) nodes were used no such effect was observed, and concluded firstly that the added lymphoid tissue acted not as a source of an immune response but as an antigen, and secondly that enhanced mice are capable of an immune response

The substance or substances responsible for enhancement have not been isolated There is some evidence however (Kandutsch, 1957, Kandutsch and Reinert-Wenck, 1957) that there are at least two components, one a protein and the other a carbohydrate, possibly combined in the form of a mucoprotein

The observations we have been considering throw much light on the nature of enhancement but it will be convenient to defer consideration of this matter until experiments with homotransplants of normal tissues have been considered

Observations with Transplants of Normal Tissues

Allen, Williams, Lovingood and Ellison (1952) found that the life of large (16 sq cm) homografts of skin in rabbits was increased from the normal seven days in control animals to 23 days in animals which had received, prior to grafting, a series of intravenous, intraperitoneal and subcutaneous injections of material prepared from the skin of the prospective donor by a process of mechanical disintegration followed by freezing and thawing

Kaliss and Spain (1952) injected mice of one inbred strain with lyophilized tissue from another, and also mice of a mixed strain with lyophilized tissue from mice of the same mixed strain Later,* each recipient received either an orthotopic skin transplant, or a subcutaneous transplant of adult spleen or embryo tissue, from the corresponding lyophilized tissue donor or an animal isogenic therewith Survival of the subcutaneous transplants appeared to be facilitated, though the effect was slight compared with that which occurs with tumour transplants The skin transplants, it is reported, "did not survive," but it is not clear what this means, for the all-or nothing criterion sometimes applicable to tumour transplants cannot usefully be applied to homotransplants of normal tissue Billingham *et al* (1956a, c), however, have investigated the matter further and have reported that the survival of skin homotransplants can be increased by a few days, and occasionally doubled, by a prior course of five injections at weekly intervals of 10 50 mg lyophilized donor strain tissue (kidney, spleen or liver) They also obtained some effect with a single injection of 100 mg on the day the skin was transplanted, but found, as expected from the work with tumour transplants, that injection of lyophilized tissue into mice which had already been immunized against homologous skin did not result in enhancement nor did it prevent the immunity from exercising its full effect

Billingham *et al* also had some success in prolonging the survival of skin homotrans

*The interval is not stated

plants in rabbits by *prior injection of lyophilized* tissue from the same—but not from a different—donor, as also did Woodruff and Simpson (unpublished) in rats, but drew attention to the difficulties encountered in attempting to assess factors which have only a small effect on transplant survival by means of experiments in genetically unstandardized animals.

Parkes (1956a, 1957a, 1958) found that the survival of interstrain ovarian homografts was prolonged in rats which had previously been injected intraperitoneally once or twice with a suspension of ovarian tissue from the donor strain. It appeared to make little difference whether, after the suspension had been prepared in a glass homogenizer, it was frozen and thawed, subjected to ultrasonic vibration, or used without further treatment.

More difficult to interpret are the observations of Hardin and Werder (1955), who found that the survival of skin homotransplants among genetically heterogeneous mice could be prolonged for long periods and often permanently by injecting saline extracts of mouse skin before, or on [illegible] transplantation. The effect was [illegible] than any reported by other [illegible] and is surprising in view of the [illegible] as the authors state, "there was [illegible] between the source of skin used as a homograft or the skin prepared as the extract for injection. It seems desirable therefore to suspend judgment on the results pending confirmation from other laboratories.

The Nature of Enhancement

The evidence presented strongly suggests that enhancement depends on the presence of specific antibodies which have either been produced in the host in response to the injection of preparations of donor (or donor strain) tissue, or else have been produced in some *other* animal in response to the same kind of stimulus and *transferred* passively in serum. The tissue preparations used to procure enhancement, as Billingham *et al.* (1956c) have pointed out, have all been subjected to treatment calculated to destroy the T antigens which determine the development of resistance to homotransplants but not the H antigens which evoke the formation of haemagglutinating antibodies, and it seems likely therefore that it is the presence of haemagglutinating antibodies of this kind which determines the state of enhancement.

How do these antibodies act?

Billingham *et al.* (1956c) have pointed out that an immunological reaction may be interfered with at three levels: afferent, central, and efferent.* Enhancement, they argue, cannot be due to efferent inhibition because it cannot be induced in animals which are already immunized, and Mitchison's failure to abolish enhancement by adoptive immunization with *normal* lymph node cells makes it appear unlikely that central inhibition is responsible; they suggest therefore that the inhibition is afferent and that the antibodies evoked by the H antigens can combine with and inactivate the T antigens as they issue from the transplant, thus delaying the onset of transplantation immunity.

Kaliss (1957) and Kaliss and Bryant (1958), on the other hand, postulate a form of efferent inhibition† in which the antibodies somehow bring about an adaptive change in the transplant in consequence of which it can survive in an otherwise hostile environment.

They give four main reasons for rejecting the hypothesis of Billingham *et al.*, and in

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*According to their definition an afferent inhibition takes effect by a direct inactivation of antigens, or by preventing their release from a graft or their access to a seat of response; a central inhibition is one which affects the machinery of antibody formation or of some equivalent immunological process, and an efferent inhibition is one which prevents the effectors of the immune reaction from exercising their action.

†*Kaliss does not use this terminology.*

the author's view these are decisive In the first place, as Kaliss (1957) showed, the passive transfer of antibodies in serum as long as 10 days *after* tumour transplantation may result in the survival of transplants which would otherwise have been destroyed Secondly, a tumour which is itself enhanced can be effectively antigenic when re transplanted to a second host of the same strain (Kaliss and Bryant, 1958) Thirdly, as Gorer (1957) has shown, a homograft in a mouse which has been injected with specific antiserum capable of causing enhancement evokes a histiocyte response which, in the early stages, is indistinguishable from that in a control mouse Finally, according to Kaliss (1957), a first graft may show evidence of enhancement due to previous injection of an antiserum while a second graft is in process of rapid destruction apparently owing to the high degree of immunity evoked by the first.

Little can be said as yet about the nature of the postulated change in the graft, but this is no reason for rejecting the hypothesis Some investigators appear to have closed their minds to the possibility of adaptive change in homotransplants, but the evidence presented earlier (p 96), in conjunction with that of Kaliss, points strongly to the conclusion that this can occur

Anomalous Response to Second or Later Homotransplants

Several instances have been reported in which, following the destruction of one or more homotransplants, the capacity of the host to react against a further transplant of the same tissue appears to have been impaired

Observations with Tumour Transplants

Lewis and Lichtenstein (1936a, b) made repeated transplants of the same mouse tumour to a single recipient belonging to a resistant strain, and found that eventually, after 12 or more transplants had failed, the tumour survived and grew progressively in the foreign strain

Kaliss (1955b), working with a mouse sarcoma ("Sarcoma I") indigenous to the A strain but which normally regressed after about 12 days in a subline of C57 black mice (designated C 57 BL/6KS), found that a second transplant to a host belonging to this subline, made one to five months after a previous transplant had regressed, would grow progressively He showed subsequently (Kaliss, 1957, Kaliss and Bryant, 1958) that if the interval between the two transplantation operations was less than two weeks the second transplant was invariably destroyed, but if the interval was longer than two weeks a proportion of second grafts survived Moreover, as the interval was lengthened from two weeks to two months the proportion of surviving transplants rose steadily, reaching about 30 per cent* when the interval was three weeks, 50 per cent* when it was one month, and 100 per cent* when it was two months

Intraperitoneal injection of antiserum, obtained from either rabbits or C 57 BL/6KS mice immunized with saline homogenates of either normal splenic tissue from A strain mice or lyophilized Sarcoma I tissue, at the time of the second transplantation modified the results With the heterologous antiserum some second transplants survived when the interval between the two transplantations was only one week, and the proportion increased to over 90 per cent when the interval was four weeks The mouse serum produced a similar but less striking effect, the proportion of surviving transplants increasing from zero when the interval between transplantations was two weeks to about 80 per cent when the interval was four weeks

When another A strain tumour (15091 a) was transplanted to C 57 BL mice the

* These figures, and those quoted in the next paragraph have been read off approximately from graphs published by Kaliss (1957)

sequence of events was the same as with Sarcoma I except that the period of resistance was longer. On the other hand when Sarcoma I was transplanted to C3H mice the hosts became and remained solidly immune

These findings are analogous to those discussed earlier under the heading of enhancement, and strongly suggest that the phenomenon under consideration is dependent on the same mechanism.

Observations with Transplants of Normal Tissues

Werder and Hardin (1954) have reported that repeated transplantation of skin in heterozygous mice from different donors to the same recipient may result in very prolonged survival of the later transplants The time interval between successive transplantations appeared to be the main factor which determined the results. When the interval between first and second transplants was 30 days each transplant was rejected within 10 days A third transplant made after a further interval of 30 days sometimes survived for as long as 24 days, and a fourth transplant made 30 days after the preceding transplant regularly survived for the duration of the experiment (10 months). When the interval between successive transplants was increased to 60 or 90 days even the second and third transplants survived for the duration of the experiment.

These results are so remarkable, and would if confirmed have such important biological and surgical implications, that it seems wise to suspend judgment about them until the experiments have been repeated in other laboratories

The Billingham-Sparrow Phenomenon

[illegible]

the survival of skin homotransplants in rabbits was increased by prior intravenous injection of a suspension of dissociated epidermal cells from the prospective donor. In their initial experiments the injection often resulted in sudden death, but it was found later that this did not occur if the epidermal cells were washed before injection. Washed cells alone were however much less effective in inducing tolerance* than unwashed cells, and eventually the following regime, which proved both safe and effective, was adopted. Each animal received a primary injection of 10-15 million washed cells, followed by a second injection 12 days later containing the same number of cells but differing in that about one quarter of the cells were unwashed. The animals were tested with skin homotransplants 22 days after the primary injection and in 9 out of 10 animals these survived from 16 to 22 days as compared with the normal survival time of not more than 9 days The weakened response of a host as a consequence of its pretreatment was found to be persistent and extended to a second transplant from the same donor; indeed in some animals these second transplants survived longer than normal first grafts. In most animals prolongation of transplant survival appeared to be due to a delay in the time of onset of a normal homotransplant reaction, but in others the histological pattern of the reaction was modified so that it appeared to be directed primarily against the graft dermis.

The phenomenon is not merely the nonspecific outcome of pretreatment of a host with epidermal cells as such, because it was found that the survival of skin homotransplants was not prolonged in rabbits pretreated with their own epidermal cells.

Injection of epithelial cells which had been killed by freezing and thawing was ineffective. Injection of viable cells intraperitoneally was also ineffective and intra-

*Billingham does not use the term tolerance in this context but states that his phenomenon "is obviously a candidate for inclusion in that category of specifically abrogated immunological response that includes 'enhancement' "

dermal injection elicited a typical immune response.

Prolongation of survival of skin homotransplants was also observed after intravenous injection of donor blood in which the number of leucocytes was of the same order as the number of epidermal cells known to be effective, but Billingham was unable to define a regimen of pretreatment with blood which gave consistent results.

Billingham writes as if it had been established beyond doubt that the effect of the cell injection is specific in that only homotransplants from the same donor show prolonged survival, and it would be surprising if this were not the case, but he does not cite any experimental data bearing on this point.

Induction of Tolerance After Irradiation

As we have seen total body irradiation may render an animal tolerant of homologous, and even of heterologous, haemopoietic cells, but there is nothing specific about this. If however a radiation chimera (p. 99) would accept other homotransplants from the original haemopoietic tissue donor, but not from other donors possessing different transplantation antigens, it could properly be said to exhibit specific immunological tolerance.

There is now no doubt that a radiation chimera will accept transplants of donor (or donor strain) tissues, and there seems no reason to doubt that the tolerance is specific although no determined attempt appears to have been made to obtain absolute proof.

The first experiments of this kind were performed by Main and Prehn (1955), who showed that skin homografts from mice of one strain survived in mice of another strain which had been irradiated and then injected with bone marrow from a hybrid of the two strains. The significance of this observation was somewhat obscured by the fact that skin homografts survived also in 2 out of 31 members of the second strain which had been irradiated and had then received isologous marrow; further experiments by Trentin (1956, 1957a) however have confirmed that mice repopulated with homologous marrow after irradiation are tolerant of donor-strain skin but have revealed no evidence of tolerance in mice repopulated with isologous marrow.

Trentin (1957a) has shown further that homografts of Cb strain* skin, which had been established for about six months on CBA mice previously made tolerant by irradiation and repopulation with Cb strain marrow, break down within two to three weeks if the recipient is given an intraperitoneal or subcutaneous injection of a cell suspension prepared from the spleen or lymph nodes of normal CBA mice. It is thus clear that the process of adoptive immunization can operate in this situation.

*Balb/c without milk factor.

CHAPTER 8

Heterotransplants

THE USUAL FATE OF HETEROTRANSPLANTS

Transplants of Normal Tissues

Heterotransplants of living tissue are, as a rule, destroyed even more rapidly than homotransplants and, despite some claims to the contrary, there does not seem to be a single well-authenticated case of permanent survival. Dead heterologous tissue is usually absorbed but may sometimes persist for months or even years.

Glandular Tissues and Skin

Ribbert (1905) transplanted human and guinea pig skin subcutaneously to rabbits. He found that the transplants were destroyed within three days, and attributed this to athrepsia (p. 67).

Leo Loeb and his colleagues (Loeb and Addison 1909, 1911; Loeb, 1920*a*, *b*, 1945) made an extensive study of heterotransplants of skin, thyroid tissue and kidney tissue interchanged between various animals including rats, mice, guinea pigs, rabbits, dogs, cats and pigeons. They found that the transplants usually became completely necrotic within 6 to 11 days, and occasionally they were destroyed much earlier. Mitotic activity diminished rapidly, but did not entirely cease until the transplant was almost completely destroyed. The ability to survive when transplanted back to the donor species was investigated for skin grafts only, and was found to persist for only 2-5 days.

Polymorphonuclear leucocytes accumulated in large numbers in and around the transplants. These cells did not appear until the transplant was partly or wholly necrotic, and Loeb therefore concluded that they were attracted by dead rather than living tissue. Their presence could not be attributed to the inadvertent introduction of micro-organisms with the transplant, because they accumulated just as readily in and around transplants of boiled heterologous tissue. There was usually intense fibroblastic proliferation around the transplant at an early stage, followed later by invasion of the transplant.

Heterotransplants, like homotransplants in immunized hosts, showed little or no evidence of vascularization, and ischaemia appeared to play an important part in bringing about their death.

There is now no doubt that these observations are substantially correct. As we shall see when we come to discuss in detail the transplantation of endocrine tissues (Chapter 23) and gonads (Chapter 24), it has been reported that heterotransplants of these tissues may survive and function for long periods, but in no case has convincing histological proof of survival been obtained. Usually tests of functional activity have been taken as evidence of graft survival when in fact the findings could be explained either by the effect of hormones liberated from a dead graft or by the activity of host tissue. Histological evidence has been put forward

in the special case of orthotopic ovarian heterografts (Chapter 21) but is unconvincing because the findings can be explained by regeneration of host ovary from capsular remnants

Bone

The fate of bone grafts is a very controversial topic which will be discussed at length in Chapter 18 As far as heterografts are concerned they certainly do not survive It has been claimed that they may stimulate osteogenesis and thus facilitate the healing of bone defects (Hughes, 1913), but other investigators have found no evidence of this (Gallie and Robertson, 1919)

Cartilage

Loeb and his colleagues (Loeb and Harter, 1926 Loeb, 1915) found that heterotransplants of living cartilage in experimental animals survived longer than heterotransplants of skin and glandular tissues They were however nearly always destroyed within three weeks, and never survived permanently In these experiments adjoining fatty tissue was transplanted with the cartilage the host reaction, which was predominantly fibroblastic, was apparent first in the fat, but later the cartilage too was invaded and replaced by fibrous tissues Lymphocytes accumulated in the surrounding tissue, but not to any great extent in the transplant itself

Transplants of dead heterologous cartilage may persist (they cannot be said to survive) much longer than living transplants, and ox cartilage preserved in formalin (Stout, 1933) or merthiolate (Wardill and Swinney, 1947, Gillies and Kristensen, 1951 Braithwaite and Hopper, 1952, Gibson and Davis, 1953) has been used quite extensively in reconstructive surgery.

The clinical value of this material will be discussed in Chapter 19 As regards its biological properties Gibson and Davis (1953) found in patients and volunteers that some absorption always occurred, first by surface erosion and fibrous replacement, later by liquefaction of the cartilage Nevertheless, thick transplants having a small surface area in proportion to their bulk sometimes persisted for many years, apparently because before surface erosion and fibrous replacement had progressed very far a thick avascular fibrous capsule formed which limited further absorption

Cornea

Kissam (1844) transplanted cornea from a young pig to the eye of a man blinded by a dense corneal scar Vision was improved temporarily but after a fortnight the graft became opaque Following this there was as Rycroft (1954) has said, "a plethora of dismal failures in corneal graft [i e hetero graft] surgery "

It would be wrong, however, to conclude that corneal heterografts inevitably fail to provide functional recovery In the first place it may well be, as Rycroft has suggested, that infection and poor technique contributed to the early failures Secondly, von Hippel (1887) succeeded in one patient, after several failures, in restoring vision for at least a year with a corneal heterograft from a young pig Finally in recent times, Babel and Bourguin (1952) and Choyce (1952) have shown that cornea from cattle, horses, pigs and sheep may remain clear when grafted to the eyes of rabbits They made the further interesting observation that second grafts from the same or a different species rapidly became opaque, although the cornea of the grafted eye remained clear

These findings are consistent with the hypothesis that under appropriate conditions corneal heterografts are gradually replaced by host tissue without the cornea becoming opaque, but that in immunized animals the replacement process is interfered with in some way and opacity results.

Blood Vessels

As we have seen, homotransplants of

blood vessel segments provide a framework which enables the vessel to be permanently reconstituted by host tissue. The work of Carrel (1907a, 1908a, 1912a) and Guthrie (1907a), who reported functionally successful heterotransplantation of blood vessel segments many years ago, suggests that the same may be true of heterotransplants

Frozen and freeze-dried heterologous vessel segments appear to offer a greater chance of success than living heterotransplants. Thus Sautot and his colleagues obtained good results in horses and dogs (Sautot, Bost and Touraine, 1952), and also in a proportion of patients (Sautot, Bost, Touraine, Martin and Feroldi, 1954), with transplants from cattle which had been frozen to −70° C. Hufnagel (1954) found that 85 per cent of freeze-dried heterotransplants of calf, lamb and pig arteries in dogs were successful, whereas fresh heterotransplants often failed and he and his colleagues have also reported four successful heterotransplants of freeze-dried calf or pig arteries in man (Hufnagel, Rabil and Reed, 1954); and Pate (1954) f[illegible]und that freeze-dried trans[illegible] [illegible] pig [illegible]ta were functionally effec[illegible] [illegible]gs in [illegible]7 out of 40 cases. On the [illegible]nd Gauthier-Villars and Oudot [illegible] that calf vessels stored for pe[illegible] [illegible] 2[illegible] days by Gross' method (p. 13[illegible]) [illegible] transplanted to dogs were less sat[illegible]isfac[illegible] [illegible] homotransplants and soon showe[illegible] [illegible]cation of the media and other changes

Tumour Transplants

Subcutaneous and intramuscular heterologous tumour transplants in normal hosts may grow actively for a short time, especially if the donor and host belong to nearly related species and the tumour is a vigorous one, but eventually they are destroyed. It is sometimes possible however to maintain a tumour by Ehrlich's (1907) method of zigzag transplantation, that is by retransplanting it back to the species of origin, then to the host species, then back to the species of origin, and so on; or alternatively, by retransplanting it at frequent intervals in the foreign species.

Putnoky (1930, 1938, 1940), using the second of these methods, succeeded in maintaining the Ehrlich mouse carcinoma in rats for many years. He made the very interesting observation (Putnoky, 1938) that the original tumour would not take in rats which had previously received transplants of normal or neoplastic mouse tissue. On the other hand when the tumour had been maintained in rats for several years, without having been transplanted back to mice, it was found to have changed in character so that it could be transplanted to other rats much more readily than the original mouse tumour.

The behaviour of tumour heterotransplants in special sites, and in animals conditioned by irradiation or administration of cortisone, will be considered later.

THE REACTION IN THE REGIONAL LYMPH NODES

Craigmyle (1958) transplanted guinea pig costal cartilage to the pinna of the rabbit and found that the regional lymph node increased in weight by from 80 to 400 per cent. The increase was present by the fourth day after grafting and was still apparent on the twelfth day when the experiment was terminated. Histologically the affected node showed cortical hyperplasia associated with the presence of many "very large pyroninophilic lymphoblastic cells."

SYSTEMIC EFFECTS

Heterotransplant recipients typically develop a polymorphonuclear leucocytosis, which with heterotransplants of normal tissue reaches a maximum a few days after transplantation, and is followed by a lymphocytosis which is usually maximal about two weeks after transplantation (Blumenthal, 1939)

BEHAVIOUR OF HETEROTRANSPLANTS IN SPECIAL SITES

Tumour heterotransplants may escape destruction, and grow until they kill the host, if transplanted to certain special sites including the anterior chamber of the eye, the brain, and sometimes the testis, of young or adult animals of various species the cheek pouch of the hamster, and the yolk sac or the chorio allantoic membrane of the chick embryo Heterotransplants of embryonic tissue may also survive for a long time in some of these sites, but this does not hold good for heterotransplants of normal tissue from non embryo donors

The Anterior Chamber of the Eye

Tumour Transplants

Zahn (1881) transplanted human malignant tumour tissue heterologously to the anterior chamber of the rabbit eye His transplants did not survive for long, but in 1913 Hegner reported that heterotransplants of mouse tumours to the anterior chambers of rats, guinea pigs and rabbits grew for a time in most cases, though they usually regressed later Hegner's attempts to grow human carcinoma and sarcoma transplants in the eye of the rat were in the main unsuccessful, but subsequently Smirnova (1937) reported that heterotransplants of a human mammary carcinoma grew, and survived for some months, in the eyes of rats, guinea pigs and rabbits

More recently H S N Greene and his colleagues (Greene, 1938, 1941a, b, 1942a, 1946, 1947, 1949, 1950, 1951b 1952, 1953b, Greene and Lund, 1944 Shrigley, Greene and Duran Reynals, 1945 Greene and Murphy, 1945 Albrink and Greene, 1953) have carried out extensive investigations from which Greene concluded that benign tumours and malignant tumours at an early stage in their life history, could not be transplanted heterologously, but that any tumour which had acquired the capacity to invade and metastasize could be transplanted to the anterior chamber of a suitable host, *i e* a host having the same type of vitamin C metabolism as the donor Once established in the anterior chamber a tumour could often be maintained by serial transplantation within the foreign species and after several passages could sometimes be transferred to sites other than the eye, especially the testis (Greene and Lund, 1944) Some tumours during their period of residence in the anterior chamber become more differentiated and in consequence it was sometimes possible to determine their origin when this could not be decided on simple histological examination of the tumour prior to transplantation (Greene and Lund, 1944)

This work is important firstly because it served as a reminder that the distinction between benign and malignant neoplasms depends on their behaviour *in vivo*, and that this cannot always be predicted correctly from purely histological data and secondly because it paved the way for more efficient methods of maintaining tumours in heterologous hosts (p 152) Greene's generalizations have, however, not been fully accepted by other investigators In the first place it is common experience that in the absence

of special treatment some tumours simply will not grow when transplanted heterologously to the anterior chamber, and with others only a small proportion of transplants are successful (Dyer and Kelly, 1946, Schilling, Snell and Favata, 1949; Morris, McDonald and Mann, 1950).* Secondly, as Greene himself recognized (Greene and Arnold, 1945 Krementz and Greene, 1953), human glioblastomas do not metastasize but can nevertheless be transplanted successfully to the eye of the guinea pig Finally, the rule that the host must have the same type of vitamin C metabolism as the donor, which implies *inter alia* that the guinea pig is the only small laboratory mammal suitable for growing human tumours, is by no means universally true.

Greene (1953b) has suggested that most if not all of the failures to obtain successful heterotransplants of tumours capable of metastasis are due to the presence of an excessive amount of stroma in the transplants The stroma, referred to by Greene as "desmoplastic" connective tissue, dies after transplantation, and in so doing, if it is present in considerable quantity, "it appears to inhibit the growth of transplantable tumour cells". One conceivable explanation of this phenomenon is that the stroma is antigenically more effective than the truly neoplastic tissue and evokes a state of immunity which destroys the tumour cells before they have become established Whatever the mechanism it certainly seems likely that the amount of stroma is an important factor in determining the fate of tumour heterotransplants, but it can scarcely be the only one.

*Dyer and Kelly (1946) transplanted human malignant tumours to the anterior chambers of guinea pigs and obtained growth with only one out of four, moreover the successfully transplanted tumour grew in only 4 out of [illegible] guinea pigs Schilling Snell and Favata (1949) transplanted carefully selected fragments from thirty six proved human cancers to the anterior chamber of [illegible] eyes in 218 guinea pigs, they obtained growth with only [illegible] tumours in a total of 29 eyes and even when growth occurred it was sometimes delayed for as long as four months Morris McDonald and Mann (1950) transplanted 40 malignant human tumours to the anterior chamber in 167 guinea pigs, and all except one failed to take

Embryonic Tissue

Greene (1943, 1947) reported that heterotransplants of mammalian embryo fragments and organs (excluding liver) survived in the anterior chamber of the eye, and could be serially transplanted within the host species. Tissue from human and rabbit embryos grew well in the eyes of guinea pigs and mice, and in one experiment was still thriving 18 months later after 4 passages in guinea pig hosts.

More recently Albrink and Greene (1953) have shown that transplants of chick embryonic tissue grow readily in the anterior chamber of the eye of the rabbit, guinea pig and mouse On the other hand mammalian embryonic tissue transplanted to the eye of the chicken evokes a pronounced lymphocytic reaction and is soon destroyed. It has also been shown by Gurchot, Krebs and Krebs (1947) that normal human trophoblast grows readily in the rabbit eye, and can be serially transplanted.

Normal Non-embryo Tissue

The rule that heterotransplants of normal non-embryo tissue are rapidly destroyed appears to hold good even when the transplants are made to special sites such as the anterior chamber of the eye. Thus Turner (1937) found that ovaries and testes, transplanted from young and new-born mice to the eyes of castrated male and female rats, evoked violent leucocytic reactions, and were either completely absorbed or replaced by fibrous tissue.

The Brain

Tumour Transplants

Shirai (1921), and subsequently Murphy and Sturm (1923), found that transplantable mouse tumours grew actively when transplanted heterologously to the brains of rats, guinea pigs and pigeons, provided, accord-

ing to Murphy and Sturm that the transplant lay entirely in brain substance. Subcutaneous and intramuscular transplants to the same animals were rapidly destroyed as were intracerebral transplants which were in contact with the ependymal lining of the ventricles. Harde (1928a, b) too found that the brain was a favourable site for tumour heterotransplants.* He attributed this to the following factors: the consistence† of cerebral tissue, the free blood supply in the brain, the slow development and relatively feeble nature of the defence mechanism, and possibly also the chemical nature of brain.

More recently Greene (1951a, 1952, 1953b, c) has shown that human malignant tumours as a rule grow to a larger size, and evoke less stroma formation, in the brains of mice and guinea pigs than in the anterior chamber of the guinea pig eye. The ability of human tumour heterotransplants to grow in the mouse brain is especially interesting in view of the fact that they seldom survive in the eye in this species.

Embryonic Tissue

Albrink and Greene (1953) found that transplants of chick embryonic tissue grew readily in the brains of rabbits, guinea pigs and mice but, as in the case of transplants to the anterior chamber, transplants of mammalian embryonic tissue did not survive in the brain of the chicken.

The Cheek Pouch of the Hamster

The cheek pouch of the hamster appears to be a relatively favourable site for tumour heterotransplants (Lutz, Fulton, Patt and Handler, 1950; Lutz, Fulton, Patt, Handler and Stevens, 1951; Lemon, Lutz, Pope, Parsons, Handler and Patt, 1952; Chute, Sommers and Warren, 1952). As we shall see later, the best results are obtained in cortisone treated recipients, but Handler (1956) showed that a lymphatic leukaemia transmissible in DBA/2 mice grew when transplanted to the cheek pouch of the hamster, whether the host received cortisone or not. Initially growth was a little slower in the non-treated hosts but on repeated transfer the growth rate increased progressively in untreated hosts and declined in hosts receiving cortisone. After the fifth passage in untreated hosts the tumour usually filled the cheek pouch in 10 days and soon after this ulcerated, as a result of which the animals died of secondary infection. Microscopically in the first 21 hamster passages the tumours resembled lymphomas and there was only limited local invasion, but in the 22nd passage one of the hamsters developed generalized leukaemia, and in all subsequent passages one or more hamsters developed generalized leukaemia within 19 days of transplantation.

Subsequently Handler and Adams (1957) showed that various neoplasms from C3H and C57BL mice could be maintained in unconditioned hamsters.

The Chick Embryo

Tumour Transplants

Murphy (1913) appears to have been the first to transplant tumours heterologously to the chick embryo. He found that transplants of a rat sarcoma to either the chorio-allantois or the body of the embryo grew readily and that the tumour could be maintained by serial transplantation. Transplants of a rat carcinoma and a human sarcoma also grew in these sites but not as readily as the rat sarcoma. Later Murphy (1914a) found that heterotransplants would not take and previously established transplants began to regress when the embryo was 18 or 19 days old. He attributed this [illegible] fundamental change in the embryo

* As we have seen (p. 57) Harde found that tumour [illegible] transplants fared no better in the brain than in the [illegible]. It is difficult to [illegible] in view of the [illegible] growth of [illegible] transplants in the brain.
† La consistance pâteuse du cerveau qui permet la [illegible] surface initiale avec peu de [illegible]

which he called the development of individuality; it may well be, however, as Sandstrom (1932) and Oakley (1938) have suggested, that transplants fail in embryos aged 18 days or more because the circulation of the chorio-allantois at this stage is degenerating

Further demonstrations of the ability of tumour heterotransplants to grow on the chick chorio-allantois have been provided by Stevenson (1917a, b, 1918) using mouse tumours, Schreck and Avery (1937) using rat and rabbit tumours, Jacoby, McDonald and Woodhouse (1943) using a mouse sarcoma, Kaufman, Prieto, Mason and Kinney (1952) using human tumours, and Heim and Schechtman (1955) using the Brown-Pearce carcinoma of the rabbit.

Taylor, Hungate and Taylor (1943) transplanted tumours heterologously to the yolk sac of the chick embryo with some success. More detailed studies (Hungate, Taylor and Thompson 1944) showed, however, that survival and growth occurred only when the cells of the transplant made contact with chick mesoderm

Embryonic Tissue

It was shown by Murphy (1913), and has since been confirmed by many other investigators (Hiraiwa and Willier, 1927, Dantschakoff and Gagern, 1929, Nicholas and Rudnick 1933 Oakley 1938), that heterotransplants of mammalian embryonic tissue may survive and grow on the chorio-allantoic membrane of the chick embryo

Non epithelial tissues, such as cartilage and connective tissues survive more readily than epithelial tissues

Nicholas and Rudnick (1933) found that with rat embryo heterotransplants the degree of differentiation which occurred depended on the stage of development at the time of transplantation. Early complete embryos underwent progressive development,* and if transplanted with the embryonic membranes intact might form a functional placenta on the chorio-allantois. On the other hand whole embryos, or large parts of embryos, transplanted when development had progressed to the stage of visible primordia (eye, ear, limb bud), retrogressed and degenerated.

Effect of Homologous or Autologous Reticulo-endothelial Tissue

It was shown by Murphy (1912, 1913, 1914a, c) that tumour heterotransplants which normally grew on the chorio-allantoic membrane of the chick embryo were rapidly destroyed if a small piece of adult chicken spleen or bone marrow was transplanted at the same time in juxtaposition with the tumour, or even to another part of the membrane (Murphy, 1914c).

Subsequently Murphy and Sturm (1923) found that various mouse tumours which could be successfully transplanted to the brains of rats, guinea pigs and pigeons—but not to subcutaneous or intramuscular sites in these animals—were rapidly destroyed if they were transplanted to the brain in juxtaposition with a small piece of autologous, but not homologous,† splenic tissue.

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*The period of observation was usually only two to five days.

†It seems likely that homotransplants of spleen do not survive long enough in the brain to be effective, but as yet this does not appear to have been proved. It may well be, also, that they do not always survive sufficiently long on the chorioallantois, because Stevenson (1917a, b) was unable to confirm Murphy's finding.

THE SECOND-SET PHENOMENON

Heterotransplants are, as a rule, destroyed so rapidly that it is difficult to demonstrate a clear cut difference between the period of survival of first and second transplants. This difficulty, however, does not arise with heterotransplants of special tissues such as

cartilage, or with heterotransplants in special sites such as the anterior chamber of the eye, and it has been shown that such transplants do induce a state of increased resistance in the host

With cartilage the power of inducing resistance appears to be retained after preservation in merthiolate Thus Gibson and Davis (1953) found that second and subsequent transplants of merthiolate preserved ox cartilage in man were absorbed more quickly than initial transplants In these experiments the transplants were all obtained from the same animal when on the other hand second transplants were obtained from a different source they behaved like first transplants Gibson and Davis concluded that the immunity induced by a transplant is either specific for cattle of the same breed as the donor or is actually individual specific The facts presented however do not provide sufficient grounds for this conclusion, and in the author s opinion it is unlikely to prove to be true because antigenic differences between individual cattle must surely be small in comparison with the difference between cattle and human beings

With tumour heterotransplants in the anterior chamber of the eye both the eye containing the transplant and the opposite eye become resistant to further transplants (Schilling and Snell 1918 Rambo Fuson Hatton and Fichwald 1951)

Snell and Favata (1951) investigated the time relations of the process and found that resistance was demonstrable about a week after transplantation to either the eye or a subcutaneous site In the light of this finding they have criticised the suggestion of Woodruff and Woodruff (1950) that homotransplants survive in the anterior chamber because immunity develops slowly, but it would seem unjustifiable to argue in this way from tumour heterotransplants to homotransplants of normal tissue

There have been conflicting reports regarding the development of immunity in the anterior chamber following heterotransplantation to a subcutaneous site In the experiments of Snell and Favata the eye became resistant if anything a little sooner after subcutaneous transplantation than after transplantation to the opposite eye Rambo *et al* (1951), on the other hand, found that subcutaneous tumour heterotransplants did not give rise to increased resistance in either the eye or the brain They attributed this surprising result to the fact that the subcutaneous transplants were destroyed very quickly but the explanation does not seem very convincing

Another illustration of the immunizing effect of a transplant is provided by the curious observation (McCartney 1922) that an animal may be rendered temporarily sterile by repeated injections of heterologous spermatozoa but here we would seem to be dealing with an antibody which is cell specific because McCartney found that homologous spermatozoa were also effective

ANTIBODIES EVOKED BY HETEROTRANSPLANTS

Heterotransplants evoke antibodies which can be demonstrated without difficulty by a variety of tests both *in vitro* and *in vivo*, and which appear to play an important part in the process of graft destruction

Tests for Agglutinins, Haemolysins and Complement fixing Antibodies

The production of haemagglutinins and haemolysins by injecting red blood cells from one animal to another of a different species is a familiar laboratory procedure

The injection is usually made subcutaneously, intraperitoneally or intravenously, but Hektoen (1911) found injection to the anterior chamber of the eye was also effective.

Steinberg and Martin (1944, 1946) reported that antilymphocytic serum, prepared by injecting lymphocytes from an animal of one species into an animal of a different species, agglutinated donor lymphocytes *in vitro*. Woodruff and Forman (1951), using rabbit-anti-rat serum, were unable to confirm this, however, nor could they demonstrate any damage to lymphocytes incubated in antilymphocytic serum, with or without added complement, by the method of unstained cell counts (p. 86). Cruickshank (1941) claimed that antilymphocytic serum also gave a positive complement fixation test when mixed with lymphocytes of the donor species.

Cooper (1957) made repeated injections of suspensions of finely divided bovine cartilage [illegible] rabbits over a period of at least a year [illegible] total dose corresponding to 1.5 g. [illegible] of cartilage. The sera of some [illegible] of the rabbits gave positive re[illegible] complement fixation tests performed [illegible] the cartilage suspension, and Cooper [illegible] he concluded that heterologous [illegible] weakly antigenic.

W[illegible] [illegible]gous organ extracts are us[illegible] the antibodies formed may be [illegible] or organ-specific. Thus He[illegible] and Groupé (1941) found that antibodies evoked by pancreatic tissue components gave complement fixation with similar material derived from other tissues of the donor species, whereas antibodies evoked by cerebral tissue components reacted similarly with cerebral tissue components from various species but not with material obtained from other tissues. Furth and Kabat (1941) found that rabbit-anti-mouse kidney serum reacted with human and rabbit kidney besides mouse kidney, though rabbit-anti-human-kidney serum did not react with mouse, rabbit, fowl or bovine kidney; and Simonsen (1953b), also working with kidney antigens, found that rabbit-anti-dog-kidney serum gave positive complement fixation tests with both dog and rabbit kidney, although the rabbits providing the serum were apparently not adversely affected by the antibodies it contained.

It is important to remember in assessing these findings that unless special precautions are taken the complement fixation test, as Maltaner (1950) has pointed out, is open to error owing to the anti-complementary effect of so called accelerator globulin which is present in many sera (especially rabbit sera).

In Vitro Tests for Cytotoxic Antibodies

Normal and neoplastic tissues may be cultured in the plasma of many, but not all, alien species. Lambert and Hanes (1911b) established this principle for a rat tumour and normal rat spleen,* and nowadays it is common practice to use heterologous plasma and heterologous embryo extract as a constituent of tissue culture media. It has been found, moreover, that tissues of two different species may be cultured together in the same flask. This was shown by Roffo (1926) with chick and rat tissues, and by Harris (1943b), and Grobstein and Youngner (1949), with rat and mouse tissues. Leone (1946) has reported that explants of chick embryo heart may be cultured with duck and mammalian embryo heart, and that cellular contact is established between the explants, causing them to beat synchronously. Murphy (1914c) found that rat sarcoma could be grown in chicken plasma in the presence of chick connective tissue, kidney and liver, although it was destroyed in the presence of chick spleen.

*Lambert and Hanes found that these tissues grew well in mouse and guinea pig plasma. Rabbit plasma was less satisfactory, and dog and pigeon plasma were still less so, but some growth occurred in all of these. No growth occurred in goat plasma.

Plasma from heterotransplant recipients, on the other hand, unlike plasma from homotransplant recipients (p 87), is as a rule* not tolerated by cultures of donor tissues. This was first reported by Lambert and Hanes (1914c), who showed that explants of a *mouse sarcoma*, which grew vigorously in normal rat plasma, were rapidly destroyed in plasma from rats which had previously received heterotransplants of the tumour, and similarly that explants of a rat sarcoma would grow in normal guinea pig plasma but not in plasma from guinea pigs immunized by heterotransplants of the tumour.

This work has been confirmed and extended, and it has now been shown that cytotoxic sera,† demonstrable by the tissue culture test, may be prepared by injecting a wide variety of heterologous tissues, including normal spleen and bone marrow (Foot, 1912, Pomerat and Anigstein, 1944), heart (Cracium and Sorescu, 1931; Harris, 1948), kidney (Verne and Oberling, 1932), and brain (Grunwalt, 1949), embryonic tissue (Lambert, 1914, Niven, 1929, Sigurdsson, 1940), and tumour tissue (Lumsden, 1924, 1925, 1926, 1931, 1937, Harris, 1943a; Imagawa, Syverton and Bittner, 1954b, and others).

Many investigators, beginning with Lambert (1914), have studied the specificity of cytotoxic sera evoked by heterologous tissue. In many instances the serum was found to be active against various tissues of the donor species in addition to the one used for immunization, and sometimes it proved to be active against tissues from other species. Some of this work must now be considered in detail.

*Goldstein and Youngner (1949) found no cytotoxic effect with serum from mice immunized by injections of heterologous kidney brei.

†The term cytotoxin (*cytotoxine*) was introduced by Metchnikoff (1899) to denote serum active against cells.

Normal Tissue (Embryonic or Adult) Used as Antigen. Lambert (1914) showed that plasma from guinea pigs immunized by heterotransplants of rat embryo skin was toxic for explants not only of this tissue but also of tumour tissue, and that serum from guinea pigs which had received transplants of rat embryo skin or injections of rat blood was toxic for explants of both rat sarcoma and rat embryo skin. Similarly, Niven (1929) found that the serum of rabbits which had been repeatedly injected with minced mouse embryos, when mixed with complement, inhibited the growth of both normal and neoplastic mouse tissues in culture, and caused cell death when added to actively growing cultures.

Cracium and Sorescu (1931) found that rabbit anti dog heart serum was active against dog embryo heart, and also to a less extent against various other dog tissues, and against heart from other species.

Sigurdsson (1940) immunized rabbits with chick embryo tissue, and tested the serum, before and after absorption with various chick tissues, on cultures of chick heart. Unabsorbed serum, and serum absorbed with chick erythrocytes, proved to be cytotoxic, but after absorption with various other chick tissues the serum ceased to be cytotoxic, it thus appeared that the operative species specific antigen was absent from the erythrocytes.

Pomerat and Anigstein (1945a, b) found that reticulo endothelial immune serum (REIS), prepared by immunizing animals* with heterologous reticulo endothelial tissue, was active against both normal (Pomerat and Anigstein, 1944, 1945a, b) and neoplastic (Pomerat, 1945) tissues from the donor species.

In contrast to the rather broad spectrum antibodies so far considered Verne and Oberling (1932) found that the serum of

* The following sera were prepared rabbit anti-chick, rabbit anti rat rabbit anti guinea pig, goat anti rabbit, goat anti-cow and goat anti human

rabbits immunized with rat kidney was effective against rat kidney only, and did not damage cultures of other rat tissues or of kidney from other species. These investigators also made the interesting observation that autolysed kidney was a more effective antigen than fresh kidney

Tumour Tissue Used as Antigen. The formation of antibodies following heterologous tumour transplantation was investigated in a long series of experiments by Lumsden. His early experiments led him to conclude (Lumsden, 1924, 1925, 1926) firstly that to produce a cytotoxic serum it was necessary to make repeated injections of the heterologous tissue, and secondly that the cytotoxin was specifically anti-cancer rather than anti-donor tissues Thus Lumsden reported that serum from rats and rabbits immunized with mouse tumour tissue was toxic for explants of the tumour but not for explants of heart, kidney and spleen from young mice (Lumsden, 1925), and that the serum of sheep inoculated with a human mammary carcinoma was lethal for explants of mouse mammary carcinoma (Lumsden, 1926 Pybus and Whitehead (1929), on the other hand, found that serum from rabbits immunized in this way was distinctly toxic for mouse heart and kidney; and that the serum of a rabbit immunized against a human carcinoma was no more toxic than normal rabbit serum for explants of mouse carcinoma

In the light of later experiments Lumsden (1931, 1937 modified his hypothesis. While admitting that heterologous tumour transplantation *might* result in the formation of antibodies toxic for normal tissues from the donor species, he still claimed that it always resulted in the formation of "anti-malignant" immune substances having no species specificity. Thus the serum of a rabbit repeatedly inoculated with mouse mammary carcinoma might contain three types of antibody: (a) natural heterotoxins which occur, though perhaps in smaller amounts, even in unimmunized rabbits, (b) anti-mouse species-specific antibodies, and (c) "anti-malignant" antibodies. The anti-malignant antibodies were difficult to demonstrate because they were masked by the other antibodies, or because they were very labile and so liable to be missed unless the serum was tested within a few minutes of its being obtained, but Lumsden claimed that they could be separated by fractionation of the serum because they were present in the "euglobulin" fraction and the species-specific antibodies occurred mainly in the "pseudoglobulin" (Lumsden, 1931; Lumsden and Macrae, 1934).

Lumsden (1931, 1932) claimed further that anti-malignant serum had some effect *in vivo*. Subcutaneous injection to animals bearing transplantable tumours was without effect, but injection to the tumour itself, provided the blood supply to the part was occluded for two or three hours, caused the tumour to regress. It is difficult to draw any conclusion from this experiment, however, because the ischaemia alone may have damaged the tumour.

Even in its modified form Lumsden's hypothesis is not fully accepted by most investigators, and the findings thought to indicate the existence of anti-malignant antibodies cytotoxic for malignant cells in general have not been confirmed by other investigators (Ludford, 1933, 1934).*

Imagawa, Syverton and Bittner (1954b) studied the rather special case of immunity to mouse mammary carcinoma. They found that the serum of guinea pigs immunized with this tumour, or with normal mammary tissue from mice bearing the mammary carcinoma virus (but not other mice), severely damaged the tumour cells in tissue culture, but failed to damage cultures of embryonic mouse intestine or of a human carcinoma.

* According to Ludford the cells which migrate from tumour explants, and which Lumsden assumed were neoplastic, are in fact stromal, and therefore normal, cells

Snell and Favata (1951) applied the tissue culture test to the aqueous humour as well as the serum of heterotransplant recipients. They found that cytotoxic antibodies could be demonstrated in the aqueous humour from each eye and in the serum seven days after heterotransplantation of a mouse carcinoma to either the anterior chamber of the eye or the subcutaneous tissue of the guinea pig.

The Neutralization Test

The principle of the neutralization test and the way in which it is performed have already been described (p. 90).

The test was used by Green (1946), who showed that the serum of rabbits immunized by heterotransplants of mouse mammary carcinoma was cytotoxic for the tumour cells.

Imagawa, Syverton and Bittner (1950, 1951, 1954a) repeated and extended Green's experiments.* They found, as Lambert and others had claimed, that normal rabbit serum (unlike normal chicken or guinea pig serum) was cytotoxic for mouse mammary cancer cells, but not to the same extent as the serum of rabbits which had been immunized with heterotransplants of the tumour.† The cytotoxic factor in normal rabbit serum was destroyed by heating to 56° C. for half an hour, and was not subsequently restored by the addition of complement.

The serum of guinea pigs immunized with mouse mammary carcinoma or with normal mammary tissue from mice bearing the mammary carcinoma virus was cytotoxic for the tumour cells, but the serum of guinea pigs immunized with normal mammary tissue from mice not bearing the virus was not cytotoxic.

These experiments constitute a rather special case because it would seem that the mouse mammary carcinoma virus was the effective antigen, but Dulaney and Arnesen (1949), and Werder, Kirschbaum, MacDowell and Syverton (1952), have demonstrated a similar phenomenon with a transplantable mouse leukaemia. Serum from rabbits which had been immunized with splenic cells or cell components (including mitochondria and whole nuclei) from leukaemic mice gave a positive test. Serum from rabbits immunized with normal mouse spleen, on the other hand, showed only a slight effect, but this may well have been due merely to a difference in titre.

In Vivo Effects of Antibodies Evoked by Heterotransplants

Diffusion Chamber Experiments

Algire, Weaver and Prehn (1957) found that lung rudiments and epidermal cells from mouse embryos survived in cell-impermeable diffusion chambers (p. 114) for at least a week without evidence of injury in the peritoneal cavity of normal rats but were killed or injured after two days in rats previously immunized by heterotransplants of mouse tissue. In other experiments they placed HeLa cells (derived originally from a human squamous cell carcinoma and maintained in culture) in diffusion chambers in normal mice and transferred the chambers five days later to older mice, some of which had been immunized against HeLa cells. The chambers were removed after seven days in the second host and the cells were found to have been destroyed if the second host had been immunized but not otherwise.

It was concluded from these experiments that heterotransplants evoke the formation of cytotoxic antibodies which are capable

*In performing the neutralization test Imagawa *et al.* exposed the cells to the serum for 3 hours at room temperature followed by 3 hours at 4° C., and then injected them into a susceptible mouse.

†This is contrary to the negative results obtained in experiments of a similar kind by Law and Malmgren (1951), but according to Imagawa *et al.* Law and Malmgren used too little serum in proportion to the number of cells used in the test.

of passing through a cell-impermeable membrane of the kind used in the experiment, and that these antibodies are capable of destroying heterotransplants even in the absence of host cells

Experiments with Cytotoxic Sera

Serum from animals immunized by heterotransplants, or in some cases by injections of heterologous tissue extracts, may produce characteristic changes when injected to a member of the donor species. We shall consider two important examples.

Nephrotoxic Serum. Masugi (1933, 1934) injected rabbit kidney extracts into ducks, and rat kidney extracts into rabbits. He then injected serum from the recipients into rabbits and rats respectively, and found that after a few days these animals developed acute glomerulonephritis which closely resembled human glomerulonephritis in its symptomatology and morbid anatomy. This observation has been confirmed by many workers, and it has also been shown that nephrotoxic sera may be produced by using as antigen certain heterologous tissues other than [illegible], notably lung (Pressman and Eisen, [illegible]) and aorta (Strehler, 1951).

Many attempts have been made to elucidate the mechanism responsible for Masugi nephritis.

Sarre and Wirtz (1942) showed that if the artery to one kidney was compressed for 15-25 minutes after intravenous injection of nephrotoxic serum this kidney did not develop nephritis, and if both renal arteries were so compressed neither kidney developed nephritis. They concluded firstly that kidney antibodies fix rapidly, and secondly that other tissues besides the kidney are capable of binding such antibodies. Pressman and his colleagues (Pressman, 1949; Pressman, Eisen and Fitzgerald, 1950), using nephrotoxic serum containing globulin labelled with radioactive iodine (I^{131}), have confirmed these conclusions, and have shown further that much more antibody becomes fixed to the glomeruli than to other parts of the kidney.

Why does the nephritis not occur until several days after injection of the serum? [illegible] .oxic [illegible], but that following injection of the serum the recipient develops anti-donor-serum antibody which combines with the donor globulin-nephrotoxic antibody complex bound to the kidney, and thereby injures the kidney. Simonsen (1953b) has modified this hypothesis by postulating that the glomerular cells themselves form, or as he puts it, attempt to form, the anti-donor-serum antibody and suffer damage in the process. The glomerular cells, he says, are compelled "to establish a defence which in itself is noxious to them." In support of this view Simonsen has pointed to the appearance of numerous pyroninophilic cells in the affected glomeruli.

Anti-leucocytic and Anti-reticular Cytotoxic Serum. Various investigators, including Ledingham and Bedson (1915) and, more recently, Chew, Stephens and Lawrence (1936) have prepared antisera active *in vivo* mainly against polymorphonuclear leucocytes by using as antigen exudates rich in these cells. Chew *et al.* injected rabbits repeatedly with guinea pig cells obtained from the peritoneal exudate after intraperitoneal injection of aleuronat, or a mixture of beef extract, gum acacia and saline. The rabbits were bled nine days after the last injection, and the serum thus obtained was heated to 56° C. to destroy complement, absorbed with guinea pig erythrocytes, and then re-heated to diminish anti-complementary activity. Parenteral injection of the serum to guinea pigs caused total, or almost total, disappearance of polymorphs from the peripheral blood,* but only a slight fall in the lymphocyte count. There was no accu-

*Five minutes after intracardiac injection; 1-7 hours after intraperitoneal injection.

mulation of polymorphs in the tissues and from this it was concluded that they had disintegrated

Later Chew and Lawrence (1937) prepared anti lymphocytic serum by making repeated injections of suspensions of guinea pig lymphocytes† to rabbits As before the serum was heated to 56 C to destroy complement it was then absorbed with guinea pig and sheep erythrocytes (the latter in order to remove Forssman antibodies) and finally re heated to 56 C to diminish anti complementary activity Intraperitoneal injection of the serum to guinea pigs caused a sharp fall in the blood lymphocyte count usually accompanied by a rise in the poly morph count This observation has been confirmed by Cruickshank (1941) and similar results were obtained by the author (p 101) using rabbit and rat serum prepared in the same manner

After repeated injection of either anti polymorph or anti lymphocytic serum the recipient becomes unresponsive and further injections have little effect Thus in the author s experiments it was found that daily injections of serum kept the lymphocyte count at a low level for about 9 days after which a sharp rise occurred in every case (Woodruff unpublished) The explanation of this phenomenon is uncertain it has been suggested that the recipient animal develops anti antibodies but Chew Stephens and Lawrence (1936) have rejected this explanation

The mechanism by which anti lymphocytic serum causes a fall in the leucocyte count has not been fully elucidated It is conceivable that this fall is in part a stress phenomenon mediated by an adrenocortical mechanism but this is not the whole explanation because the phenomenon occurs though possibly in lesser degree in adrenalectomized animals (*see* Woodruff and Forman 1951) To demonstrate this small doses of serum had to be used because injection of the usual dose was rapidly fatal in adrenalectomized animals

Serum somewhat akin to anti lymphocytic serum has been prepared using as antigen minced spleen lymph node and bone marrow it is termed anti reticular cytotoxic serum (ACS) (Bogomolets 1943 Straus 1946) or reticulo endothelial immune serum (REIS) (Pomerat and Anigstein 1945a)

Many remarkable properties have been claimed for this serum by the Russian workers In experimental animals large doses of heterologous ACS are said to decrease and small doses to increase the resistance to tumour transplants and the rate of healing of fractures (Bogomolets 1943) Clinically small doses of rabbit anti human ACS are said to be of value in such diverse conditions as osteomyelitis lung abscess puerperal sepsis disseminated sclerosis schizophrenia peptic ulcer eczema and inoperable malignant disease it is also said to promote the healing of wounds and fractures (Bogomolets 1943 Marchuk 1943 Linberg 1943) *

The reports of the clinical value of the serum lack confirmation and are regarded with scepticism by most medical men outside Russia It is of interest however that Straus Horwitz Levinthal Cohen and Runjavac (1946) confirmed in rabbits that small doses of ACS (goat anti rabbit) stimulated fracture healing and large doses retarded healing

The specificity of ACS was studied by Straus Runjavac Zaitlin Duboff and Swerdlow (1946) They titrated the serum by means of a complement fixation test against antigen derived from the donor species used in preparing the serum and also against antigen from other species and found that it was almost entirely species specific The serum contained both haemag

† [illegible]

* [illegible] see Straus (1946)

glutinins and haemolysins, the former in higher titre than the latter; but these could be selectively absorbed without materially altering the ACS titre.

NON-SPECIFIC MODIFICATION OF THE REACTION TO HETEROTRANSPLANTS

In this section we shall consider the effect of various procedures including irradiation, administration of cortisone, thyroidectomy and hypophysectomy on the behaviour of heterotransplants.

Tumour Transplants

It was shown many years ago by Murphy (1914b) that heterotransplants of mouse tumours survived longer in rats pre-treated by exposure to X-rays than in normal rats.

More recently the problem of growing *human tumours in animal hosts* has aroused much attention. Transplantation to special sites (p. 141) in itself has not proved sufficiently reliable but the problem has now been to a large extent solved, owing mainly to the work of Toolan and her colleagues. This began with the observation (Toolan, 1951) that human tumour tissue survived for 8 to days after subcutaneous transplantation to irradiated rats and mice Further investigations by Toolan and others (Toolan, 1953, 1954a, b, c, Patterson, Chute and Sommers, 1954, Patterson and Patterson, 1956, Handler, Davis and Sommers, 1956; Herbut and Kraemer, 1956; Pierce, Verney and Dixon, 1957) have established that many human tumours will grow, and that some can be propagated by serial subcutaneous transplantation in weanling rats conditioned by irradiation, administration of cortisone, or both, and by transplantation to the cheek pouch of hamsters conditioned by cortisone *

Toolan (1957b) has also succeeded in maintaining human tumours in cortisone-treated mice of certain strains, notably DBA, LAF, Swiss-Webster and, to a less extent, AKM.

The factors which determine success or failure in experiments of this kind have not been fully elucidated. One curious finding is that successful transplants sometimes grow more vigorously in the experimental animal than in the human donor, and may kill the host in as short a time as ten days.

The effect of endocrine ablation on tumour heterotransplants was investigated by Schatten and Bergenstal (1958). They found that a mouse ascites tumour grew (in the sense that tumour cells and ascitic fluid could be demonstrated in the peritoneal cavity 5 days later) in 98 out of 120 hypophysectomized rats, and in 34 out of 50 rats made hypothyroid with I^{131}, but in only 25 out of 170 untreated controls.

Transplants of Normal Tissues

Toolan (1954b) reported some success with heterotransplants of human embryonic tissue in cortisone-treated animals. More recently Adams (1958) has shown that heterografts of mouse skin survive up to 70 days in hamsters treated with cortisone for six weeks (to a total dosage of 30 mg.) whereas they are rejected after two weeks in untreated hamsters.

Another promising line of investigation which is attracting a good deal of interest at the present time is the study of heterotransplants of haemopoietic tissue in ani-

*In these experiments irradiation, when employed, was given in a dose of 150r prior to transplantation. Rats receiving cortisone were given 3 mg at the time of transplantation and on the second, fourth and sixth days thereafter Hamsters received 3 mg. cortisone at the time of transplantation and this was sometimes followed by 1.5 mg seven days later Serial transfer was usually carried out at weekly intervals

mals subjected to total body irradiation. *This* work will be discussed in detail in Chapter 22. In brief it has been shown that survival of mice following otherwise lethal *irradiation can* be prolonged by heterotransplantation of bone marrow from rats or guinea pigs, and in some experiments the surviving animals have been shown to be chimeras. *Usually* however a severe form of secondary disease (p. 157) develops and only rarely does the life span of the treated animals approximate to normal.

SPECIFIC IMMUNOLOGICAL TOLERANCE OF HETEROTRANSPLANTS

Induction of Tolerance Before the Development of Immunological Maturity

Hašek and his colleagues *joined embryo* birds of *various species by the technique of* embryonal parabiosis (Hašek, 1953a, b). Evidence of partial tolerance to erythrocytes of the partner's species was obtained *in some experiments after* chicken-turkey (Hašek, 1954; Hašek, Hraba, Benešová and Hlaváčková, 1955; Hraba, 1956), and pheasant-chicken (Hašek, 1954) parabiosis, including in one instance hetero-chimerism *in a hen* persisting for eight weeks following embryonal parabiosis with a turkey embryo (Hraba, 1956).

After chicken duck parabiosis Hašek and his colleagues (Hašek, 1954; Hašek, Hraba, Benešová and Hlaváčková, 1955; Hašek and Hraba, 1955a) found that the ducks became partially tolerant to chicken erythrocytes but the chickens subsequently reacted normally to duck erythrocytes and duck skin. Billingham, Brent and Medawar (1956a) on the other hand found that chicken-duck parabiosis resulted in a considerable degree of *mutual tolerance to each other's skin,* though never in anything like complete tolerance. Test transplants became vascularized and survived for periods ranging from 8 to 15 days, though even the best of them appeared chronically swollen and hyperaemic, whereas heterografts from ducks to untreated chickens or to a treated chicken from a duck other than its parabiotic partner did not become properly vascularized and became completely macerated or mummified within 6 days.

Injection of heterologous whole blood to embryos has proved less effective than embryonal parabiosis but has yielded some positive results.

Simonsen (1955) injected chick embryos *intravenously with* blood from adult turkeys, and turkey embryos with blood from adult chickens. Natural agglutinins occur in the serum of each of these species against red blood cells of the other two but are not present before the age of 1 to 6 weeks. He found that the average titre of natural antibodies against turkey blood was significantly lower in 5 week old chicks that had been injected with turkey blood as embryos *than in untreated controls,* and at *10* weeks of age the sera of two out of twelve treated birds still contained no anti-turkey agglutinins although they contained antibodies to goose blood cells, of which the birds had had no embryonic experience. In addition chicks which had been injected as embryos with turkey blood, and turkeys which had been injected as embryos *with chicken* blood, showed a diminished antibody response when given a second injection of the same blood 5 weeks after hatching. It was therefore concluded that some degree of tol*erance to turkey blood* can be induced in chickens and *vice versa.* Similar experiments in which chick embryos received goose blood yielded much less clear-cut results, and Simonsen attributed this difference to the *fact that chickens and turkeys* are taxonomically more closely related than chickens and geese.

Hašek and his colleagues found that 13

day chick embryos injected intravenously with turkey, guinea fowl or duck blood failed to develop any tolerance to erythrocytes or other blood constituents of the donor species (Hraba, Hašková, Lengerová and Vojtíšková, 1956), and intravenous injection of duck and guinea fowl blood to 15 day goose embryos and hen blood to 15 day guinea fowl embryos was equally ineffective (Hašek, 1956). On the other hand intravenous injection of goose blood to 15 day duck embryos resulted in partial tolerance, as shown by diminished capacity to form agglutinins and precipitins when the birds were later challenged with blood of the donor species (Hašek, 1956).

Puza and Molnár (1956) injected rabbit erythrocytes to rat embryos 1 to 5 days before birth and later, when the recipients had attained a weight of between 40 and 100 g., challenged them with a further injection. They found that their capacity to form agglutinins was significantly impaired.

Repeated post embryonal intravenous injection has proved surprisingly effective (Hašková and Pokorná, 1956). The degree of tolerance produced with a given species combination seems to depend not only on the amount of antigen injected during the adaptive period but also on the length of time over which it can act during this period. Hašková's (1957) most remarkable results were produced in ducks which developed lifelong inability to form hetero-agglutinins to goose or hen blood following a course of 15 intravenous injections of 0.3 to 0.7 ml. whole blood on alternate days, starting within six days after hatching; curiously, however, these birds showed no tolerance to heterografts of donor skin.

Egdahl and Varco (1957) found that rats which as embryos received an intraperitoneal injection of a cell suspension prepared from mouse spleen or lymph node became, if they survived, partially tolerant of mouse skin, as shown by the fact that grafts became vascularized and survived between 11 and 18 days, whereas rats injected intravenously at birth did not become tolerant at all.

It seems clear from all this work that methods which induce a high degree of tolerance to homologous tissue are only marginally effective when applied to more remotely foreign cells. Billingham *et al.* (1956a) have suggested that a high degree of tolerance to heterologous tissue might be achieved by exposure to foreign cells at a sufficiently early stage of embryonic life, and point to the experiments of Eastlick (1941) in which it was found that 2½ to 3½ day avian embryos of one species appeared to be highly, though not completely, tolerant of limb buds from embryos of another species.* Simonsen (1955, 1956) has reported however that the period during which some degree of tolerance to human erythrocytes can be induced in chicks is preceded as well as followed by a neutral period, and it is conceivable that this is but one example of a general rule.

As we have already seen even partial tolerance may permit a homologous tumour to kill a normally non-susceptible host. Bollag (1955), in similar experiments in which a heterologous (mouse) tumour was transplanted to rats which as embryos had received an injection of mouse liver and spleen cells, found however that while the transplants grew to a large size they eventually regressed.

Induction of Tolerance After the Development of Immunological Maturity

It has been shown by Zaalberg, Vos and van Bekkum (1957) that irradiated mice which have been repopulated with rat bone marrow will tolerate heterografts of rat skin so long as the state of chimerism persists.

*Eastlick was concerned to draw attention to the fact that the experiments revealed some degree of incompatibility whereas in the present context the significant fact is that this was not very much greater.

CHAPTER 9

Storage and Sterilization of Tissues

The problem of devising satisfactory methods for storing tissues prior to transplantation has attracted many workers, the pioneer in this field being Alexis Carrel.

The methods used may be grouped under the following headings:

1. Tissue culture, and perfusion of organs.
2. Storage at temperatures just above freezing.
3. Freezing.
4. Storage at room temperature after freeze drying.
5. Storage at room temperature after fixation by chemical agents or by heat.

Tissue growing in culture is, in Carrel's phrase, in a condition of *active life.*

Tissue stored at temperatures just above the freezing point or actually frozen may, under suitable conditions, be kept alive for a period varying from a few weeks with unfrozen tissue to a year or more with tissue frozen under optimal conditions. Such tissue has a low rate of metabolism and, in Carrel's phrase, is in a condition of *latent life.* Its viability may be demonstrated by tissue culture or by transplantation to a suitable host.

Tissue which has been freeze-dried, or fixed by chemical agents or heat, is dead.

These methods of storage must now be considered in detail.

TISSUE CULTURE

The term tissue culture is used here to denote the maintenance of fragments of living tissue or isolated living cells in a nutrient medium at a temperature at, or near, the normal body temperature of the species from which the tissue was obtained. Tissue culture was first successfully undertaken by Harrison (1907) using nervous tissue from amphibian embryos. It has become a commonplace and highly efficient procedure as a result of the work of Carrel, Strangeways, Champy, Fell, Gey, Parker, Earle and many other investigators.

Conditions for Successful Culture

The concentration of various electrolytes in the culture medium and the pH are highly critical; there must be an adequate amount of glucose; and for growth to be maintained the medium must provide an adequate supply of nitrogen, and must contain growth stimulating substances, which are usually added in the form of embryo tissue extract.

For long term culture the medium must be changed, and the tissue subcultured, at appropriate intervals.

As a rule the tissue must have access to oxygen, but embryo tissues may survive for a considerable time in the absence of oxygen by anaerobic glycolysis.

With most forms of culture a firm sub-

strate is provided for the newly formed cells in the form of a plasma clot, or a surface of glass, cellophane or other material. Until recently it was believed that such a substrate was essential but, as we shall see, Earle (1954a) has shown that it may be omitted under certain conditions.

Types of Culture

The material used to start a tissue culture may take the form of a small piece of tissue, a more or less disorganized collection of cells, or a single cell.

Culture of Small Pieces of Tissue

The usual method, which was devised by Carrel (1912a), is to embed the tissue in a plasma clot which is attached on one aspect to a glass surface and is elsewhere in contact with a fluid medium. Various techniques are illustrated in Figure 41; for a detailed description, however, the reader should consult a textbook on tissue culture such

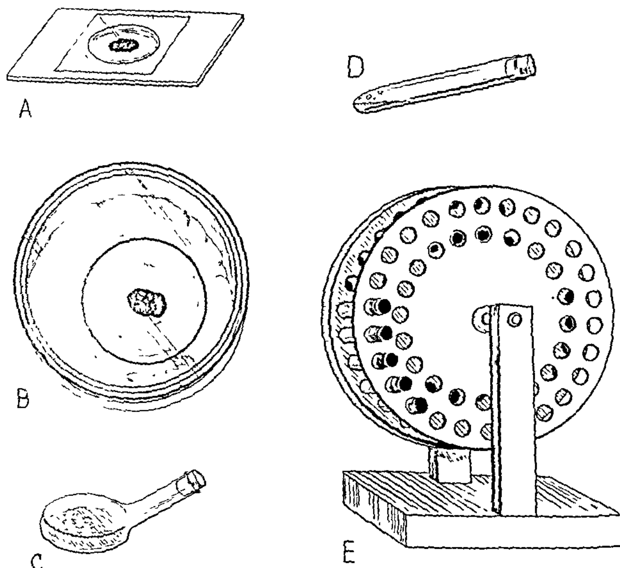


Fig 41. Some methods of tissue culture. A. Coverslip method. B. Watch glass method for culturing small organs or organ rudiments. C. Culture in Carrel flask. D, E Roller tube culture. The explants are embedded in plasma clot lining the tube (D) and bathed by fluid medium The tubes are carried in compartments in a drum (E) which is made to revolve at about 12 revolutions per minute.

as that of Parker (1950). The coverslip technique is the simplest but is inconvenient when large numbers of explants are to be cultured.

For many purposes interest centres in the cells which grow out by migration and by cell division from the periphery of the explant into the surrounding clot, and it is these outgrowing cells which are commonly used to start new cultures.

It is possible by repeated subculturing to maintain both fibroblasts and epithelial cells for many years and it seems likely that they may be propagated indefinitely in this way. Cells thus propagated may be used for transplantation, but usually when it is required to store and transplant cells as distinct from organized tissues, it is best to use cultures of cell suspensions (*v. infra*).

When on the other hand it is required to store and later transplant organized tissues as distinct from cells, a different technique, termed by Fell (1952) *organ culture** is used. Now it is the main bulk of the explant which is important, and outgrowing cells if they are present are discarded. The explants are usually cultured on plasma clots in watch glasses (Fell, 1940), though the hanging drop and other techniques may be used. Each explant is moved to a different part of the clot, or to a different clot every few days, but subculture† is not performed.

The size of explants used for organ culture is critical, and the optimal size depends partly on the tissue and partly on the composition of the medium and other conditions of culture.

With tissues having a high rate of oxygen consumption central necrosis will occur if the pieces are too large. Explants a millimetre or two in diameter are often used, but according to Earle (1951b) central necrosis is likely to occur if the diameter exceeds 0.2 mm. The size of explants must not be decreased beyond a certain limit, however, unless special precautions are taken. According to Earle (1951a) "even the best of our culture media [are] actually very poorly suited for the growth of tissue cells *in vitro* until the medium has been altered or adapted by the metabolic activity of living cells," and very small pieces of tissue are especially liable to damage on this account. The damage may be minimized, however, by culturing a small fragment in close proximity to a large one (Borghese, 1950), or by using medium taken from a rapidly growing culture (Earle, 1951a).

Explants maintained by organ culture cannot as yet be kept alive indefinitely, but material maintained in this way for a few days or weeks has been used quite extensively in transplantation experiments.

Lux, Higgins and Mann (1937a, b) cultured explants of guinea pig, rabbit and rat adrenal cortex (each about 0.5 mm diameter) in plasma clot cultures in Carrel flasks at 37.5°C for periods up to 18 days, and used the explants as homotransplants (p. 112).

Gaillard and his colleagues (*see* Kooreman and Gaillard, 1950; Gaillard, 1951) used plasma clot cultures in watch glasses for storing explants of human parathyroid* obtained from new born babies. The medium consisted of human plasma (1 part), human cord serum (2 parts), human foetal brain press juice (2 parts) and Gey's saline† (15 parts), and the cultures were incubated at 37°C. Every third day the explants were transferred to fresh medium and after being maintained *in vitro* for periods up to 60 days they were transplanted to patients suffering from post-operative tetany. In some

*The method is often used for the culture of whole organs from embryos. For a detailed description see Fell (19[illegible]).

†The term subculture implies that a portion of the explant is used to start a fresh culture.

*Stone, Owings and Gey (1934a, b) appear to have been the first to transplant cells grown in tissue culture to human beings. They too used cultures of human parathyroid but they transplanted the outgrowing cells whereas Gaillard used the whole explant.

†See Gey and Gey, 19[illegible].

cases (p. 491) clinical improvement occurred, but in no instance was any attempt made to obtain histological proof that the transplants had survived.

Martinovitch (1939, 1951a) suggested that the life of explants might be prolonged by incubating the cultures at a temperature a few degrees below body temperature (e.g. at 34 C for mammalian tissue instead of 37 C), and by using a low concentration of growth-stimulating substance (embryo extract) in the medium. In this way he kept explants of infantile rat and mouse gonads alive in watch glass cultures for several months. Explants of infantile rat pituitary were more difficult to maintain, but could be kept alive for three months if they were placed on a piece of tunica albuginea which in turn rested on the plasma clot.

Scott (1953) cultured infantile rat adrenals in a fluid medium by a more elaborate technique. The explants were supported in a mass of glass helices,* like currants in a cake, and the medium was kept circulating by means of a gas lift (Carrel and Lindbergh, 19[illegible]). This method was not very satisfactory, however, and the maximum period of survival was only 14 days.

[illegible]xplants of [illegible]dult skin, consisting of epidermis an[illegible] of the dermis, may be cultured [illegible], raw surface down, upon an aer[illegible] fluid medium as described by Med[illegible] 1948a). Proliferation and migrat[illegible] epithelium occurs, and usually results [illegible] encystment. So far no-one appears [illegible] tried to culture skin for more th[illegible] week or two by this method.

*Glass helices were first used in tissue culture work by Earle, Schilling and Shannon (1951).

Culture [illegible]trains of Cells

It is n[illegible] possible, thanks to the work of Earle and his colleagues, to culture pure strains of cells starting in each case from a single cell. The selected cell is planted in medium in a capillary tube (Sanford, Earle and Likely, 1948); by this technique diffusion is restricted, and according to Earle (1954a) this gives "the isolated cell a better chance to alter the culture medium directly adjacent, thus allowing it to build up a more optimal zone of altered or conditioned medium around itself."

If the procedure is successful and cell proliferation occurs, the culture may be transferred to a large flask* and cultured in a fluid medium under a sheet of perforated cellophane† (Evans and Earle, 1947; Earle, Evans, Edward and Duchesne, 1949).

By shaking and scraping a rapidly proliferating culture of this kind a dense cell suspension can be obtained and used to start a fresh culture; and as Shannon and Earle (1951) have shown it is possible to dispense with the cellophane substrate in the new culture and obtain luxuriant growth on the glass floor of the culture flask. The quantity of cells which can be grown in this way is surprisingly great. Earle has obtained up to 10 g. wet weight of cells at a time, and estimates that his laboratory could produce up to 100 g. per month. The cultures may be maintained without transfer for six months or more, and by subculturing they may apparently be maintained indefinitely.

The work just reviewed marked the beginning of the long-term, large-scale culture of cell strains. The next advance was the development by Earle, Schilling and Shannon (1951) of the three-dimensional culture, in which the cells are supported on a solid matrix built up of ⅛ inch diameter pyrex glass helices. Finally, Earle (1954a) succeeded in culturing cells in suspension in the absence of a solid substrate. To achieve this he used roller tube cultures

*The flasks used by Earle and Highhouse (1954) h[illegible] a floor area of 60 sq. cm.

†Cellophane substrate cultures, as they are [illegible] may also be started with cells obtained by minc[illegible] freshly obtained tissue, but this of course gives rise t[illegible] mixed, as distinct from a pure strain, culture. Ma[illegible] cultures of cells derived from chick embryo tissue h[illegible] been studied by Shannon, Earle and Waltz (1952), [illegible] by Grossfeld (1952).

rotated at speeds up to 10 revolutions per minute. The medium consisted of dilute horse or human serum and chick embryo extract with the addition of about 0.1 per cent of methylcellulose to increase the viscosity slightly. To start the culture he used a dense inoculum of cells in suspension so that rapid conditioning of the medium was achieved.

Pickerill (1951, 1952) suggested that cultures of human epidermal cells might constitute a useful source of material for homografting. One reason why surgeons have been reluctant to try this is that it has been suggested that malignant mutations may arise in the cultures. To investigate this Perry, Evans, Young, Earle and Hyatt (1957) transplanted human epidermal cells which had been derived from a single cell and maintained in culture for three years to the cheek pouch of the hamster. The transplants were quickly destroyed, suggesting that malignant change had not occurred. Even if the fear proves groundless, however, cultured epidermal cells are unlikely, in the author's view, to be of clinical value for reasons discussed in Chapter 13.

PERFUSION OF ORGANS

An isolated organ may be kept alive for a time by connecting it temporarily to the vascular system of a suitable animal, or by perfusing it with artificially oxygenated blood or an artificially oxygenated erythrocyte suspension propelled by a mechanical pump.

The first of these methods offers no prospect of long-term storage of organs. It has been used experimentally, however, to maintain the coronary circulation temporarily during homotransplantation of the mammalian heart. Marcus, Wong and Luisada (1952), who first described this procedure, have termed it *interim parabiotic perfusion*.

The second method, perfusion by a mechanical pump, was first investigated by Carrel and Lindbergh (1938), but their apparatus was quite inadequate. Many of the technical problems which defeated the earlier workers have, however, now been solved.

In the first place, infection can be controlled by antibiotics.

Secondly, blood clotting can be prevented by adding heparin and by coating with silicone all surfaces which come in contact with blood unless they are already unwettable.

Thirdly, there has been a determined effort to devise apparatus for use as an artificial heart-lung in patients undergoing operations on the open heart. As a result of this work, which is briefly reviewed in Chapter 21, and in which Gibbon, Lillehei and Kirklin in America, Bjork and Crafoord in Sweden, and Melrose in England have played leading parts, highly efficient pump-oxygenators are now available which are capable of maintaining a pulsatile flow of oxygenated blood for long periods.

It might be thought that by taking advantage of all these new developments it would be possible to maintain isolated organs alive by perfusion for days, weeks or even months. This expectation has not been fulfilled and, as Aird (1953) has reported, after some hours the perfused organ swells visibly and the outflow of blood from it progressively and rapidly diminishes, but the reason for this failure is unknown.

Another approach to the problem, which is under investigation in the author's laboratory, is to use a perfusing medium which does not contain erythrocytes or even any haemoglobin and to cause it to carry sufficient oxygen by enclosing the whole apparatus, together with the organ being perfused, in a chamber containing oxygen under pressure.

STORAGE AT TEMPERATURES JUST ABOVE FREEZING

The possibility of storing living tissues at *temperatures just above freezing** was first demonstrated by Wentscher (1903), who reported successful autotransplantation of human skin after storage in the icebox for periods of seven and fourteen days. A few years later Carrel (1907a, 1908a) reported successful homotransplantation of dog arte- [illegible]al segments which had been stored for 10 days at 0° to 1° C. in sealed glass tubes each containing a small piece of absorbent cotton moistened with Locke's solution. Subsequently Tuffier (1910, 1911) transplanted various human tissues which had been stored in petrolatum in the icebox for periods ranging from a few hours to two months and Magitot (1911) reported successful homotransplantation of human cornea [illegible] from an eye which had been stor[illegible] serum in the icebox for 8 days.

O[illegible] possibility of this method of sto[illegible] demonstrated it became impor- tant [illegible] how long different tissues co[illegible] under various conditions of [illegible] to determine the optimal co[illegible] tissue.

[illegible] this problem was Alexis Car[illegible] who studied the behaviour of va[illegible] stored at −1° to 7° C. in sealed [illegible] of them in an atmosphere of air [illegible] immersed in saline,† defibrinated b[illegible] petrolatum. He found that skin, car[illegible] and blood vessels retained their no[illegible] histological appearance after storage f[illegible] months, whereas thyroid and k[illegible] disintegrated. Skin and connective tissue could be successfully transplanted after storage for at least two weeks. Cartilage and periosteum could be successfully transplanted after storage for 18 hours, but the effect of storage for longer periods was not determined. Blood vessel segments transplanted after storage for several months often functioned satisfactorily, but Carrel was unable to determine whether the transplants survived or were replaced by host tissue. In these experiments storage in petrolatum appeared to give the best results, but the superiority of this method was not conclusively proved.

Subsequent investigators have confirmed and extended Carrel's findings, and have modified the technique in various ways for storing different tissues including, in particular, skin, bone and blood vessels.

The procedures used, and the results obtained, will be considered in detail in later chapters. In brief the tissue is kept in a moist atmosphere in a sealed jar, or alternatively is immersed in a fluid medium, of which the best appears to be a balanced saline solution with *10 per cent homologous* serum. Skin (p. 218), bone (p. 363), blood vessel segments (p. 430) and ovary (p. 495) have been shown to give good results on transplantation after storage at 0° to 6° C. for two or three weeks, and in some cases after storage for as long as two months. Viable cells have been demonstrated by tissue culture in skin (Allgöwer and Blocker, 1952) and blood vessel segments (Peirce, Gross, Bill and Merrill, 1949) after storage in this way. In addition bone has been shown to be capable of taking up P^{32} (Kiehn, Friedell, Benson, Berg and Glover, 1950) but the significance of this finding is uncertain (p. 360). [illegible] 3° C. appears to [illegible] tisfact[illegible] e as far as skin [illegible] onc[illegible] i, 1950).

*i.e. above the freezing point of the tissue fluids.

†Carrel tried three different solutions, 0.9 per cent NaCl, Locke's solution, and Ringer's solution.

FREEZING

THE BIOLOGICAL EFFECTS OF FREEZING

Nearly three hundred years ago Robert Boyle published a remarkable treatise on cold (Boyle, 1683), in which he reported among other things that extreme cold prevented putrefaction in animal tissues, and that frogs and fish were able to survive for a short time when the water surrounding them was frozen.

A century later Spallanzani (1776) observed that silkworm eggs would survive freezing at −21°C for a few hours.

Subsequent investigations have shown that viruses (Rivers, 1927), many bacteria (MacFadyen and Rowland, 1900a, b), and some unicellular and small multicellular animals and plants (Becquerel, 1905, 1936, 1950) may survive freezing down to or below the temperature of liquid air and subsequent thawing. Isolated cells and tissues from non-mammalian vertebrates, for example frog spermatozoa, frog muscle, and chick embryo heart, may also survive these procedures (Luyet and Hodapp, 1938, Luyet and Thoennes, 1939, Gonzales and Luyet, 1951), but mammalian cells, with a few exceptions, are usually killed unless special precautions are taken.

The Mechanism of Injury

Despite the collective and individual complexity of living cells, freezing in a biological matrix, as Meryman (1957) has aptly expressed it, represents nothing more than the removal of all available water and its isolation into inert foreign bodies, the ice crystals.

The deleterious effect of freezing* and thawing on living cells has been attributed to two main factors: mechanical damage caused by ice crystals, and damage due to concentration of electrolytes and other substances either within the cells or in the surrounding medium. In addition there may be incidental injury, referred to as thermal shock, resulting from changes of temperature *per se*.

Formation of Ice Crystals

Luyet and his colleagues (Luyet, 1939, 1949, 1957, Luyet and Hodapp, 1938, Luyet and Gehenio, 1940, Luyet and Hartung, 1941), while not denying that electrolyte concentration may play some part, have claimed that mechanical damage caused by ice crystals is the main factor. In their view, when cells are frozen and thawed, crystallization is apt to occur when the temperature is below freezing but above a certain critical temperature. If, however, tissue is rapidly cooled to below the critical temperature, *the protoplasm becomes vitrified*, that is to say it passes into a solid amorphous state without crystallizing, and no damage occurs. The tissue remains vitrified and undamaged if stored at a temperature below the critical level, and may safely be thawed provided it is warmed sufficiently rapidly.

It now seems clear that this is not the whole story. There is widespread agreement that when an aqueous solution is frozen the faster the rate of cooling the smaller the ice crystals, and further that recrystallization may occur in the solid state unless the temperature is maintained below a certain level, which in the case of biological materials may be as low as −70°C (Meryman, 1957). According to Meryman (1957) however when tissue is frozen slowly, for example by putting it in a conventional deep freeze at about −20°C or even by freezing in air at the temperature of solid carbon dioxide, ice crystal formation is exclusively extracellular* and does *not* cause significant damage.

*The term *freezing* is here used in a wide sense and implies that either the medium in contact with the cells is frozen or the cell protoplasm is frozen, or both.

*Intracellular crystallization may be induced during →

although with rapid freezing intracellular crystals form and are responsible for at least part of the damage which occurs.

Changes in Solute Concentrations

The possibility of damage being caused during freezing by solute concentration, first suggested by Müller-Thorgau (1880), was for a long time not appreciated except by botanists In 1929, however, Moran showed that the damage sustained by frog muscle on freezing could be accounted for quantitatively in terms of electrolyte concentration, and more recently the part played by this factor has been further defined by Lovelock (1953, 1954).

Lovelock's analysis starts from the observation that, when a dilute aqueous solution is frozen, a mesh of ice crystals is formed which is permeated by a system of channels continuous throughout the mass As freezing progresses the channels become smaller and, since ice separates out as a pure substance, the solution in them becomes more and more concentrated until, at the eutectic, all becomes solid.

The process of concentration, according to Lovelock (1957a), changes the physical environment of the cells in several different ways, all of which are potentially harmful. In the first place the ionic strength of the suspending medium is increased Secondly, the pH of the medium may change, particularly if sparingly soluble buffering salts crystallize out Thirdly, the concentration of substances such as urea or dissolved gases may be raised to toxic levels Finally, the complete removal of water may bring the cells and their structures into actual physical contact. The lipid-protein complexes which make up the principal external membrane of living cells, and probably also other semipermeable membranes bounding the nucleus, the mitochondria, the microsomes and other cell components, appear to be particularly liable to damage from these causes.

Thermal Shock

Injury resulting from a sudden change in temperature has been observed to occur in spermatozoa at temperatures well above the freezing point, and has been attributed by Lovelock (1955) to thermal expansion. When cells are frozen however it is impossible at present to differentiate between injury due to freezing and thawing and injury due to changes of temperature *per se.*

Factors Which Determine the Fate of Frozen Cells

The factors which determine the fate of frozen cells have not been fully elucidated, but the following variables have been shown to be important:

1. Cell permeability.
2. The rate of cooling.
3. Treatment with glycerol and other substances.
4. Conditions of storage.
5. The rate of thawing.

Cell Permeability

When a cell such as a fresh water amoeba, with a relatively impermeable membrane, is cooled the protoplasm becomes supercooled without changing in composition, until, at a certain temperature, it freezes. The temperature at which internal freezing occurs is usually between $-5°$ and $-10°$C., but it is conceivable that, as Heilbrunn (1943) has suggested, the cell may be cooled to lower temperatures without freezing if the protoplasm is in the gel form. Sudden cooling, according to Heilbrunn, produces protoplasmic gelation, but "if cooling is too rapid ice may form before the protoplasm has time to gel." If this is true it would seem that, as Heilbrunn claims, the danger of internal freezing would be minimized by cooling the cells rapidly down to the temperature at which gelation begins, keeping

the freezing process, as Chambers and Hale (1932) showed many years ago in experiments with frog muscle, by introducing a small crystal of ice into the cell, and this always results in cell death

the cells at this temperature until gelation was complete, and then cooling further.

When on the other hand, as with most mammalian cells, *the cell membrane* is *highly permeable to water*, the cell remains *in osmotic equilibrium* as the temperature is lowered. It thus becomes more and more dehydrated until finally it is too dry to freeze. Under these circumstances the cell is likely to be killed by dehydration long before the channels in the ice mesh have become sufficiently small to endanger it mechanically. Water-permeable cells are *able to tolerate brief exposure to hypertonic salt solutions however, and therefore, according to Lovelock*, may survive if frozen sufficiently rapidly to a temperature below −10°C., stored at this temperature, and rapidly thawed.

The Rate of Cooling

As we have seen, with slow freezing ice crystal formation is exclusively extracellular and injury results from the high concentration of solutes in the medium in which the cells are bathed during the freezing process.

With rapid freezing intracellular crystallization occurs. If freezing is sufficiently rapid the crystals may be so small as to be innocuous, but this can only be achieved with pieces of tissue which do not exceed 1-2 mm. diameter (Meryman, 1957).

Treatment with Glycerol and Other Substances

Results of great theoretical and practical importance have been obtained by studying the effect of exposing cells to the action of various substances before or during freezing.

Luyet and his colleagues (*see* Luyet and Hartung, 1941; Gonzales and Luyet, 1951) found that freezing and thawing caused less damage if the tissue (or organism) was pre-treated with ethylene glycol, and they suggested that the glycol was beneficial because it caused partial dehydration of the tissue. Some years later Keeley, Gomez and Brown (1952) carried out further investigations with this substance, using split grafts of dog skin. The grafts were soaked in ethylene glycol for periods up to three minutes, frozen either "rapidly" by being held over the surface of liquid nitrogen or "ultra-rapidly" by being immersed in liquid nitrogen, and thawed quickly in saline at 40°C. Later, to test their viability, the grafts were each transplanted back to the original donor. None of the rapidly frozen grafts survived, but the ultra-rapidly frozen grafts survived in 98 per cent of cases if thawed immediately. Grafts pre-treated with glycol, *frozen ultra-rapidly and stored at* −70°C. for up to 123 days, also survived in 59 per cent of cases, but grafts stored for longer periods failed to do so. Keeley *et al.* concluded that pre-treatment with ethylene glycol protected the cells against damage during freezing, but their evidence is not entirely convincing because no less than 86 per cent of grafts which were ultra-rapidly frozen without any pre-treatment, and immediately thawed, were found to be viable.

Pre-treatment with sugar solutions has also been tried, again with the object of

, and

Card

(1941) is effective in protecting fowl spermatozoa against damage during freezing.

Much the most effective substance is glycerol, the protective effect of which was discovered by Polge, Smith and Parkes (1949). These investigators found that fowl spermatozoa were destroyed by freezing to −79°C. in Ringer's solution but survived when frozen to this temperature, or to −196°C., in a medium containing 20 per cent glycerol. Direct microscopic observations made with a special freezing slide (Smith, Polge and Smiles, 1951) showed that ice crystals formed in the medium at a temperature between −10°C. and −20°C., but there was no evidence of internal freezing, nor did any of the crystals appear to contain spermatozoa.

A proportion of human spermatozoa may

survive freezing in solid carbon dioxide (−70°C.), liquid nitrogen (−196°C) and liquid helium (−269°C) in the absence of glycerol or other substance having a similar action (Jahnel, 1938; Shettles, 1940; Hoagland and Pincus, 1942; Parkes, 1945) provided that it is not too rapid (Polge, 1957), but Polge *et al.* found that the survival rate was higher when 10 per cent glycerol was added to the medium. Spermatozoa of several other mammalian species have been found to survive freezing to low temperatures and subsequent thawing only when treated with glycerol and, in addition, frozen slowly (Smith and Polge, 1950; Polge and Rowson, 1952, Polge, 1957).

Recovery of motility after thawing has been the usual criterion of survival, but Polge (1951) has shown that fowl spermatozoa frozen in a medium containing glycerol also retain their fertilizing power, and glycerol-treated frozen bull semen is now used extensively for artificial insemination in cattle.

The protective effect of glycerol was next demonstrated with mammalian erythrocytes It was shown first that erythrocytes could be frozen and thawed without lysis if glycerol was present in suitable concentration (Smith, 1950; Smith, Polge and Smiles, 1951) A little later it was discovered that if rabbit (Sloviter, 1951) or human (Mollison and Sloviter, 1951) blood was mixed with glycerol-saline to give a final glycerol concentration of about 15 per cent, many of the erythrocytes survived freezing to and storage at −79°C., and if, after thawing, they were freed of glycerol by dialysis, they behaved when transfused like fresh erythrocytes.

Finally, glycerol has been shown to protect various mammalian tissues, including skin (p. 166), cornea (p. 168), and gonadal (p 169) and endocrine tissue (p. 169).

The mechanisms by which glycerol, ethylene glycol and other substances exert their protective effect have not been fully elucidated. It is clear, however, that *inter alia* these substances act as diluents* and lower the concentration of salt which is in equilibrium with ice at any given temperature (Lovelock, 1954; Rey, 1957), and secondly that they reduce the speed of crystallization and the size of the crystals which are formed (Rey, 1957).

Conditions of Storage

The length of time for which tissue which survives freezing remains viable depends *inter alia* on the temperature at which it is maintained. Thus, as we have seen, rat ovary frozen in glycerol-saline does not survive for more than a few days at −79°C, but has been shown to be viable after storage for a year at −190°C. Fowl spermatozoa frozen in glycerol-saline are nearly all dead after three months at −79°C. and after 12 months at −190°C. (Parkes, 1954). Rat anterior pituitary stored for 90 days in liquid air, and subsequently thawed and explanted in tissue culture, has a very much greater delay before it begins to grow than similar tissue stored for only a few days (Smith, 1954).

It appears from these examples that tissue may undergo slow deterioration even at extremely low temperatures, and it seems likely that this is due partly to recrystallization in the solid state. In addition, as Meryman (1957) has pointed out, frozen material is not as inert as it might appear, because some of the water does not become ice but is hydrogen bonded or adsorbed to organic molecules, where it may still be available as a solvent for electrolytes and a vehicle for denaturation.

The Rate of Thawing

It is generally agreed that rapid thawing, which may be achieved by immersing the tissue in saline at 37° to 45°C., is less damaging than slow thawing (Billingham and Medawar, 1952; Taylor, 1957).

*Lovelock (1953a, b, 1954) speaks of glycerol as a *salt buffer*, but this analogy may be misleading.

PRESERVATION OF TISSUE BY FREEZING

We shall consider first the problem of freezing without loss of viability, and secondly freezing as a means of preserving tissue when viability need not necessarily be maintained

Freezing without Loss of Viability

Tumour Tissue

Many investigators, starting with Michaelis (1905) and Ehrlich (1907), have reported successful propagation of tumours by grafts of frozen tumour tissue (for review *see* Walsh, Greiff and Blumenthal, 1950) A few reports discuss the effect of variations in the rate of freezing (Breedis, Barnes and Furth, 1937, Breedis and Furth, 1938, Klinke, 1939, Mider and Morton, 1939, Breedis, 1942, Snell and Cloudman, 1943, Walsh, Greiff and Blumenthal, 1950), and these indicate that the proportion of tumour takes is higher with slowly frozen than with rapidly frozen tissue

It was suggested by Salvin Moore (Salvin-Moore and Barratt, 1908, Salvin Moore and Walker, 1908) that the successful propagation of a tumour by grafts of tumour tissue which had been frozen might depend on the transference of a virus rather than of living tumour cells, and more recently this hypothesis has been strongly advocated by Gye and his colleagues (*see* Gye, 1949, Mann, 1949a) As Gye himself expressed it, prolonged exposure of tissue to a temperature of $-79°C$ results in death of all or almost all the cells, but 'dead malignant cells are capable of starting a new, strictly new, tumour in virtue of the intrinsic virus they contain ' In support of this hypothesis it has been claimed that mincing a tumour prior to freezing damages a majority of the cells (Gye, Begg Mann and Craigie, 1949), that the activity of frozen tumour tissue, instead of decreasing during storage, actually increases (Mann, 1949a) that histological examination of frozen tumour tissue soon after it has been grafted reveals complete, or almost complete, necrosis (Gye, 1949) and that mouse mammary carcinoma can be transmitted by frozen tumour tissue only when the material is injected in the vicinity of the host's mammary gland, where, it is suggested, the virus makes contact with normal mammary tissue (Mann, 1949a)

We are not here concerned with the etiology of cancer nor with the importance or otherwise of viruses in this connexion, but it is relevant to point out that this evidence will not stand up to critical examination

In the first place it is quite possible, as Walsh *et al* (1950) have clearly shown, to mince, freeze and thaw tumour tissue without causing the gross change in cellular structure described by Gye and his colleagues This of course does not constitute proof that the cells remain viable, but it disposes of one of the arguments put forward in support of the contrary claim

Secondly, as Walsh *et al* (1950) point out, the loss of potency which Mann (1949b) herself has shown to occur with repeated freezing and thawing, 'is more consistent with the interpretation that the effectiveness of frozen material is due to the survival of tumour cells, since many infectious viruses can survive an indefinite number of such treatments '

Thirdly, as Bittner and Imagawa (1950) have shown, mouse mammary carcinoma cannot be transmitted by cell free extracts, and can be transmitted by frozen tumour tissue only to mice having the same genetic constitution as the mouse in which the original tumour arose

Fourthly, and decisively, survival and multiplication of cells in grafts of frozen tumour tissue has been conclusively demonstrated by histological examination of grafts left in situ for periods ranging from as little as two hours up to the time when palpable tumours developed (Walsh *et al* , 1950, Pas

sey and Dmochowski, 1950; Passey, Dmochowski, Lasnitzki and Millard, 1950, Warner, Gostling and Thackray, 1950)

Freezing is now being used as a convenient means of storing viable tumour tissue (*see* e.g. Klein, Révész and Klein, 1957; Toolan, Winkler-Haemmerli and Korngold, 1957, van Nie and Mühlbock, 1958). The effect of pre-treatment with glycerol and other substances has not been widely investigated, in the experience of Toolan *et al.* however it is beneficial with some tumours but with others either makes little difference or is actually harmful. These rather anomalous findings would appear to suggest that optimal conditions were not achieved.

Skin

Mider and Morton (1939) found that rat skin, frozen quickly to reach −50°C. within 20 seconds and then immediately thawed, survived in part when transplanted to a subcutaneous site. Skin which had been frozen slowly, however, survived more completely. Subsequently Briggs and Jund (1944) showed that mouse skin which had been frozen could be successfully transplanted orthotopically; and again they found that grafts frozen slowly gave better results than those frozen quickly, whereas rapid thawing was better than slow thawing.*

Strumia and Hodge (1945) found that split grafts of human skin which were placed in citrated blood, frozen slowly over a period of 15-30 minutes to −25°C., stored for periods up to 61 days, and quickly thawed, survived when transplanted back to the original donor and proved to be very nearly as satisfactory as fresh autografts. Baxter and Entin (1947, 1948), on the other hand, had no success with human skin grafts stored at −20°C. or at −70°C. although grafts stored in plasma, in saline-impregnated gauze, or in air, at −8°C. proved viable for periods up to three weeks; and Allgöwer and Blocker (1952) found that explants of human skin which had been frozen to −36°C. and stored for 14 days subsequently showed no outgrowths of either fibroblasts or epithelial cells in tissue culture.

Billingham and Medawar (Billingham and Medawar, 1952; Billingham, 1954) studied the effect of different rates of freezing and thawing on the epithelium of rabbit ear skin. With skin which had been soaked in Ringer's solution slow freezing gave slightly better results than quick freezing, whereas rapid thawing gave much better results than slow thawing. Even slow freezing and rapid thawing caused some damage, however, because when transplanted back to the original donor the skin proved to be inferior to a fresh autograft in that it regenerated a sparser hair crop and did not recover the characteristic cosmetic qualities of ear skin. The epidermal melanocytes proved, as expected, to be more easily damaged than the graft epithelium, and survived only when the grafts were slowly frozen and quickly thawed.

Skin which had been soaked in a mixture of glycerol (15 per cent) and Ringer's solution survived even quick freezing followed by slow thawing. Slow freezing followed by quick thawing was tolerated even better, and grafts so treated, and stored at −79°C. for more than a year, behaved like fresh grafts. Moreover, epidermal cells suspended in a mixture of Ringer's solution and glycerol (15 per cent) survived both slow and quick freezing, whereas epidermal cells suspended in Ringer's solution failed to survive whatever the rates of freezing and thawing.

Keeley, Gomez and Brown (1952) reported that split grafts of dog skin, not subjected to any special pre-treatment, survived

*In these experiments the tube containing the skin to be frozen was surrounded by one or two concentric tubes (depending on whether quick or slow freezing was required) with air gaps in between, and the whole assembly was surrounded by solid carbon dioxide. Quick thawing was obtained by pouring warm Ringer's solution (25°C) over the skin, slow thawing by immersing the tube containing the skin in a water bath at 30°C.

if frozen ultra rapidly by being plunged into liquid nitrogen (with vigorous agitation to prevent the accumulation of gas bubbles) and immediately thawed,* and could be successfully transplanted back to the original donor, but failed to survive if frozen more slowly by holding them tightly stretched 2 3 cm above the surface of liquid nitrogen These findings are in sharp contrast with those of Billingham *et al*, and it is difficult to account for them because, as Billingham (1954) has pointed out, it is doubtful whether the so-called ultra rapid freezing used by Keeley *et al* provided any faster cooling than the rapid freezing of Billingham and Medawar (1952) in which the grafts were flattened on to strips of copper foil and immersed in an isopentane bath cooled to −150 C

Woodruff (1959) used homografts of human skin which had been frozen slowly to −79 C in 15 per cent glycerol saline, and stored for periods up to 5 months in severely burned patients and found that they behaved like fresh homografts (Figs 42, 43)

*Grafts frozen in this way and later stored for a week at −20°C did not survive when transplanted back to the original donor



Fig. 42. Extensive deep burn one week after application of homografts of skin which had been immersed in 15 per cent glycerol saline for one hour frozen to −79°C. and stored at this temperature The grafts were applied without adequate preparation of the bed but despite this about half of them took and the patient's condition improved dramatically Autografts were used to cover the remaining defect and later to replace the homografts Healing was complete four weeks after the first grafts were applied (Author's case Reproduced from *Recent Advances in Surgery* by courtesy of J & A Churchill Ltd)

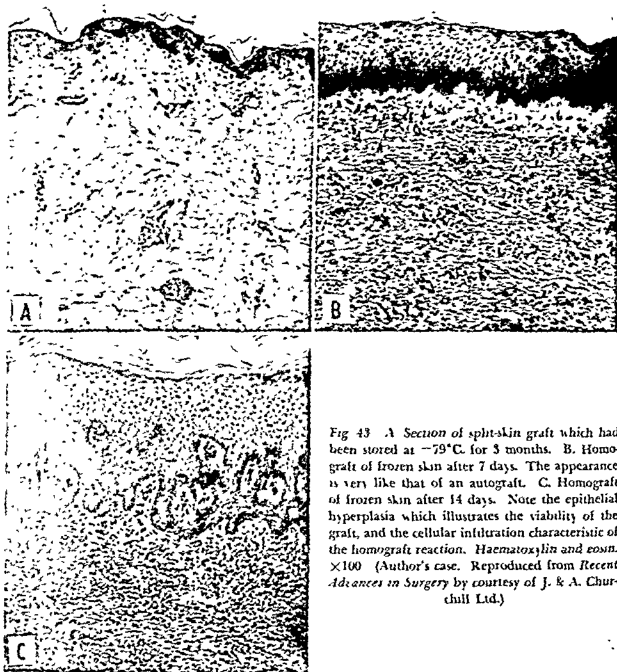


Fig 43 A *Section of* split-skin graft which had been stored at −79°C. for 3 months. B. Homograft of frozen skin after 7 days. The appearance is very like that of an autograft. C. Homograft of frozen skin after 14 days. Note the epithelial hyperplasia which illustrates the viability of the graft, and the cellular infiltration characteristic of the homograft reaction. *Haematoxylin and eosin.* ×100 (Author's case. Reproduced from *Recent Advances in Surgery* by courtesy of J. & A. Churchill Ltd.)

Cornea

Corneal grafts frozen without special pre-treatment are unsatisfactory because they become opaque a few days after grafting (Smelser and Ozanics. 1946), but encouraging results have been obtained by Eastcott, Cross Leigh and North (1954) with human corneal grafts frozen after pre-treatment with glycerol Billingham (1957b) has reported that minute gas bubbles appear during thawing in the corneas of whole eyes frozen to −79°C. after pre-treatment with 15 per cent glycerol, but these do not appear to damage the graft and they usually disappear quite soon.

The pH of the medium appears to be fairly critical and Ridge's (1957) work suggests that a pH of about 8.8 is desirable because in less alkaline media the concentration of glycerol required to prevent significant damage by freezing and thawing is in the toxic range.

Endocrine Tissues and Gonads

Thyroid and Parathyroid Blumenthal and Walsh (1950) studied the behaviour of autografts of guinea pig thyroid and parathyroid which had been stored frozen They concluded that rapid freezing in liquid nitrogen gave better results than slower freezing achieved by placing the tubes containing the tissue in solid carbon dioxide but the difference does not appear to be statistically significant and in any event the results may have been influenced by the fact that all the recipients of quickly frozen grafts were given thyrotrophin (TSH) postoperatively whereas only 3 out of 12 recipients of slowly frozen grafts were so treated

Stewart Stewart and Woodruff (1955) found that rat thyroid survived freezing to −79 C in 15 per cent glycerol saline (Fig 14) but ceased to be viable after storage for seven days at this temperature



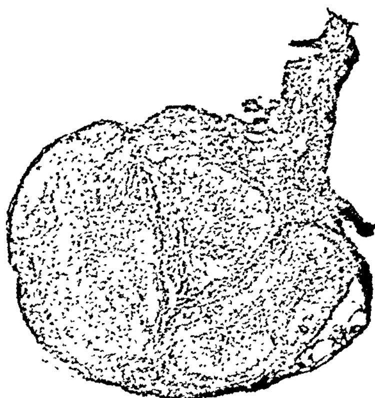
Fig 14 Autograft of thyroid of rat after 4 weeks. Before grafting the tissue was soaked in 15 per cent glycerol saline for an hour and frozen at −79 C for 24 hours.

Adrenal The initial attempts of Smith and Parkes (1954) and Rob and Eastcott (1954a) to freeze adrenal cortical tissue without loss of viability were unsuccessful despite pre treatment with glycerol saline Later however Parkes (1955) obtained functional grafts of rat adrenal cortical tissue which had been frozen slowly (in either one or two stages) in buffered Ringer Locke solution containing 15 20 per cent glycerol and stored for 24 hours at −79°C The histological appearance of one such graft is illustrated in Figure 15

Ovary Parkes and Smith (Parkes and Smith 1953 Smith 1953 Smith and Parkes 1951) found that ovarian tissue from young adult rats cooled slowly in serum to −79 C and stored at that temperature for 9 days survived when subsequently thawed and grafted subcutaneously to the original spayed donor it did not survive however if cooled in serum to −190 C Similar tissue cooled slowly in serum or saline containing 15 per cent glycerol on the other hand survived cooling to −190 C and was found to be viable after storage at this temperature for more than a year although rapidly frozen tissue did not survive and even slowly frozen tissue soon ceased to be viable when stored at −79 C

In these experiments the endocrinological activity of the graft was assessed by making vaginal smears to determine whether the oestrous cycle was restored In addition grafts were removed at intervals for histological examination The histological findings suggest that during freezing and thawing some of the ovarian cells including a large number of ova were destroyed Further destruction took place during the first 24 hours after grafting but thereafter regeneration began to occur from surviving cells a small number of oocytes being reformed * Subsequent investigations by

* According to Zuckerman (1951) neogenesis of oocytes does not occur in adult ovarian tissue and the oocytes



A

B

Deanesly (1954b, 1956) confirmed that rat ova are very sensitive to freezing and thawing but that some may survive and may subsequently undergo normal development and ovulation even in subcutaneous grafts.

In later experiments Parkes (1957b) cooled ovarian tissue slowly at the rate of about 1 C/min down to −15 C and thence faster to about −70 C in the slow cooler described by Polge and Lovelock (1952) after which the ampoules containing the tissue were placed in a mixture of alcohol and solid carbon dioxide (−79 C) or in liquid air (−190 C). With autografts or intrastrain homografts of rat ovary frozen in this way in rat serum without the addition of glycerol and stored for seven days, the proportion of takes was low and the latent interval between grafting and the reappearance of the vaginal cycle with such grafts as were successful was about five times greater than normal. Grafts of rat ovary frozen in either saline or serum containing 15 per cent glycerol and stored at −190 C for up to a year on the other hand invariably took but the latent interval was about twice normal, indicating that the tissue was extensively damaged but that enough cells survived to permit effective regeneration. For storage at −79°C 15 per cent glycerol serum proved to be a much better medium than glycerol saline.

Mouse ovary appears to be more resistant than rat ovary to freezing and thawing. Parkes (1957b) found that autografts of mouse ovary which had been frozen slowly in 15 per cent glycerol in horse serum and kept at −79 C for 2 hours invariably took and the latent interval between grafting and re-establishment of the vaginal cycle was the same as with fresh grafts, and Parrott (1958b) working in Parkes' laboratory, succeeded after a number of failures in obtaining a live litter from frozen ovarian tissue grafted orthotopically to a mouse whose own ovaries had been destroyed by X-irradiation.

Testis. Deanesly (1954a) found that subcutaneous intrastrain homografts of testicular tissue from 7 day old rats which had been frozen slowly in 15 per cent glycerol saline and stored at either −79 C or −190 C for varying periods* took readily and caused growth and secretion of the seminal vesicles of the hosts. After four to sixteen weeks the histological appearance varied but to about the same extent in frozen and control (unfrozen) grafts. In general the interstitial cells were well developed but the tubules were small and often showed only a single row of germinal epithelium and Sertoli cells.

Intrascrotal grafts of frozen testicular tissue did not take quite as well as the subcutaneous ones probably according to Deanesly, owing to vascularization difficulties, but if they did become established the tubular epithelium survived as readily as the interstitial cells, and 12 weeks after

seen in grafts of mature rat ovary represent the persistence of oocytes which were present in the tissue when grafted.

*Grafts frozen to −79°C were apparently stored for 7 days only but those frozen to −190°C were stored for 7 weeks or longer.

←

Fig. 15. Subcutaneous autografts of stored adrenal tissue in adrenalectomized rats. A. Fourteen days after transplantation of 4 entire half-adrenals which had been soaked in 15 per cent glycerol saline for 1 hour and frozen at −79°C for 24 hours. Three nodules corresponding to 3 of the half adrenals transplanted are visible each showing a lightly staining peripheral zone of rapidly regenerating cortex surrounding a core of debris (× 4). B. Forty two days after transplantation of four enucleated half adrenals which had been soaked in 15 per cent glycerol saline for 1 hour and frozen at −79 C for 24 hours. The graft was composed of three lobes parts of two of which are shown in the illustration (× 150). (Reproduced from *Proceedings of the Royal Society* by courtesy of Dr. A. S. Parkes and the Royal Society.)

grafting the tubules appeared normal and contained motile spermatozoa.

Whole Animals and Organs

Whole Animals. Over twenty years ago Russian investigators reported that they had resuscitated bats and ground squirrels cooled to temperatures below O°C. (Kalabukov, 1934; Murigin, 1937), but this work aroused little interest, at any rate in the West.

In 1951 Andjus of Belgrade announced that, by using special methods of cooling and rewarming, adult rats could be revived and could survive indefinitely after being cooled to 7-9°C., and could survive for a few days after being cooled to 1 C This report was received with a good deal of scepticism because previous workers (Ware, Hill and Schultz, 1947, Adolph, 1948) had found that rats cooled below about 15°C. did not recover spontaneously on rewarming and had concluded that they were dead. Fortunately, however, Andjus was invited to demonstrate his procedure in London at the National Institute for Medical Research, and subsequently collaborated with investigators there in improving the technique until they were able to revive rats and mice regularly after an hour at body temperatures just above 0°C. (Andjus and Smith, 1954; Andjus and Lovelock, 1955; Goldzveig and Smith, 1956).

In Andjus' method the animals were cooled in two stages: firstly confined in small containers in a refrigerator so that they were exposed to a progressive fall in oxygen tension and rise in carbon dioxide tension,* and, later, after their temperature had been reduced to about 20°C., in crushed ice. Respiration ceased below about 9°C. and the heart stopped beating at about 6°C. To revive the animals heat was first applied locally to the region of the heart and at the same time artificial respiration was started. The heart soon began to beat (Fig. 46) and when the body temperature had risen to

*It is of interest that Ware *et al.* (1947) had previously achieved the same object by wrapping the thorax and abdomen of rats and rabbits with adhesive tape sufficiently tightly to interfere with respiration, and then cooling them.

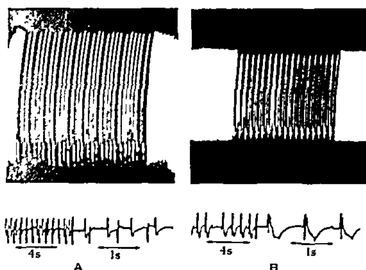


Fig 46. Kymograph tracings and electrocardiograms recorded from the heart of a hamster which had been freezing progressively for 30 minutes until the deep body temperature reached −0 7°C. A. Immediately after thawing. B Four hours later. Drum speeds 8 and 128 mm./min. (Reproduced from *Proceedings of the Royal Society* by courtesy of Dr. Audrey Smith and the Royal Society.)

about 15° C respiration became spontaneous. When this occurred the rewarming process was completed by immersing the animal in water at 40°C.

Smith, Lovelock and Parkes (1954) applied Andjus' technique to golden hamsters, which are natural hibernators, and succeeded in reviving these animals after they had been supercooled to between −3° and −5° C, and also after they had been solidly frozen for periods up to 15 minutes.

In later experiments frozen hamsters were resuscitated by warming the whole body by dielectric heating and giving artificial respiration (Smith, 1956a, b; 1957a; Lovelock and Smith, 1956). Before the animals were thawed ice was present not only in the skin and superficial tissues but also in the brain and internal organs, and calorimetric studies suggested that as much as 50 per cent of the body water had frozen in some animals which recovered fully.

Some animals which were resuscitated died soon afterwards, but the mechanism of death has not been elucidated. Vascular stasis was found to persist in various organs, including the spleen, liver and kidneys, for some hours after thawing, and small haemorrhages were sometimes seen on the surface of the lungs, but there must have been other profound changes of a more subtle kind. Billingham (1957c) has suggested that following cooling to 0° C or below the mucosa of the gut may become permeable to potentially harmful bacteria, but this has yet to be proved or disproved.

It is of interest that pregnant hamsters which had been frozen for 30 minutes with body temperatures below 0° C during the first 8 days or on the 12th day after fertilization of the egg and subsequently resuscitated, gave birth to normal litters. Those frozen on the 9th, 10th or 11th days did not give birth to young, and this was attributed to the fact that the hamster placenta undergoes rapid growth and a radical change in structure during this period, and might in consequence be especially vulnerable (Smith, 1957a, b).

The dielectric heating method, though successful with hamsters, was found to be unsuitable for larger animals because of the difficulty of obtaining a uniform field distribution which would effectively warm the animal without causing superficial burns. Lovelock (1957b) succeeded, however, in designing a diathermy apparatus* with an output of approximately 500 watts by means of which animals could be warmed sufficiently uniformly, and this has been used by Smith (1957a), in conjunction with artificial respiration, to resuscitate frozen rabbits and also prosimian primates of the species *Galago crassicaudatus agisymbanus* (Fig. 17).

The rabbits were washed in icy water containing a synthetic detergent, partly shaved, and then placed in a bath of propylene glycol kept at −5° to −6° C and stirred by jets of compressed air. The deep body temperature fell to the freezing point of plasma within an hour of cessation of respiration, and 20–40 minutes later the extremities had begun to freeze. Some of the galagos were frozen in the same way. Others were cooled by a closed box technique for 2½–3 hours, then transferred to icy water until breathing stopped at temperatures between 7° and 9° C, and finally placed in a bath of propylene glycol at −5° C to reduce the deep body temperature to below 0° C and freeze the tissue fluids.

Thawing was extremely rapid, the deep body temperature being raised from −0.6° to between 10° C and 15° C in about one minute, and thereafter at the rate of 1° to 2° C per minute. During the process cold air was blown over the surface of the animal to prevent overheating of the skin or premature dilatation of the blood vessels.

In both the rabbits and the galagos the

* Th[illegible] a[illegible]al to be warmed (F[illegible]g [illegible]) was placed in the c[illegible] of a [illegible] tuned to [illegible] Mc/s and coupled to a [illegible] at the [illegible] frequency.

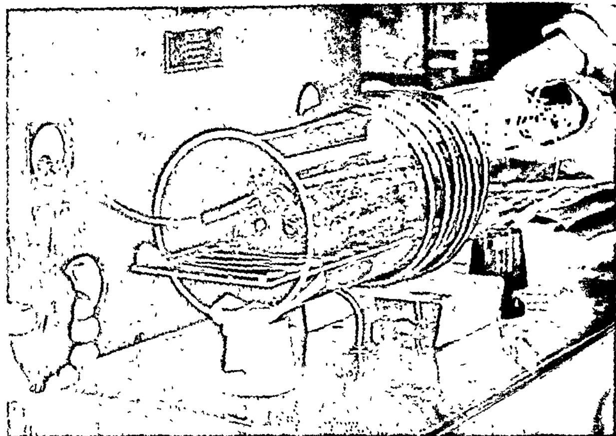


Fig. 47. Reanimating a frozen galago with diathermy. The animal lies inside a perspex tube surrounded by the output coil of the diathermy apparatus. Artificial respiration is being given by insufflating air into a tracheal cannula. (Reproduced from *Proceedings of the Royal Society* by courtesy of Dr. Audrey Smith and the Royal Society.)

heart resumed beating when the internal temperature reached about 15°C, and spontaneous respiration started when the temperature was between 20° and 30°C. Some of the animals sat up and moved around, but within an hour they all collapsed and died. At autopsy the only obvious lesion was severe haemorrhage in the upper part of the stomach, and this was thought to be due to diffusion of hydrochloric acid into the mucosa when the circulation had been arrested by cold. Neutralization of the gastric contents with bicarbonate prior to the cessation of respiration prevented the gastric haemorrhage and resulted in slightly longer survival, but all the rabbits died within 4 hours and the galagos within 24 hours.

The Isolated Heart. Smith (1957a) reported that hearts removed from hamsters which had been frozen for 30 minutes showed no impairment of function when the coronary circulation was perfused with a nutrient fluid at room temperature and gave a normal electrocardiogram. Moreover hearts from hamsters which had been frozen for 3 hours, and which had therefore passed the stage at which resuscitation is at present possible, resumed beating when perfused *in vitro* and, provided that the intra-thoracic temperature had *not* fallen below −2°C, continued to beat for several hours.

The rat heart recovered completely after freezing to −2°C for an hour, and showed slight recovery after freezing to −4.8°C, but not after freezing to −5°C.

The tortoise heart was found to be more

resistant still and survived freezing for long periods at −2 C and for short periods at −10°C

In another remarkable experiment Smith (1957a) cooled an isolated hamster heart to between 10° and 15 C and then added glycerol to the perfusing medium The concentration of glycerol was gradually increased to between 15 and 20 per cent as cooling progressed and then gradually reduced after rewarming Some of the hearts treated in this way subsequently resumed beating after being frozen to and thawed from −20 C (Fig 18)

Other Organs. So far there appears to have been no serious attempt to apply the techniques of Smith and her colleagues to other isolated organs It would be of great interest however to freeze the kidney of a large animal such as the dog, rewarm it by diathermy, and replace it as an autotransplant by vascular anastomosis

Freezing When the Tissue Need Not Remain Viable

Transplants of bone and blood vessels may give excellent results even when the tissue is dead, and for storage purposes they are therefore commonly frozen without any special precautions to maintain viability *

A satisfactory technique is to place the tissue in sterile tubes, freeze fairly slowly by surrounding the tubes with solid carbon dioxide, store the tubes in an insulated container filled with solid carbon dioxide, and thaw the grafts quickly when required by removing them from the tubes and plunging them into saline at 37°C

More rapid freezing may be achieved by immersing the tubes in a mixture of solid carbon dioxide and alcohol but although this is commonly used there is no evidence that it gives any better results Ultra rapid

*The various procedures which have been used are described in detail in Chapters 18 and 21 respectively

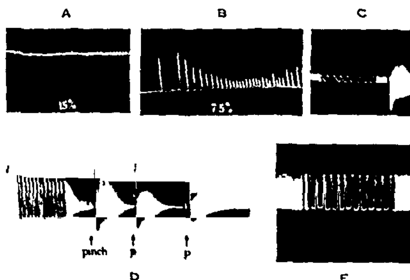


Fig 18. Kymograph tracings from the heart of a hamster which had been perfused with 15 per cent glycerol and thawed from −20°C. The glycerol was gradually removed during perfusion at +20°C. A Immediately after thawing B Glycerol concentration has been reduced to 7.5 per cent C and D Perfusion with glycerol stopped E Four hours after thawing (Reproduced from *Proceedings of the Royal Society* by courtesy of Dr Audrey Smith and the Royal Society)

freezing, by plunging the tissue directly into liquid nitrogen at −195°C., was at one time regarded as desirable, but is of no advantage, and at any rate in the case of artery grafts may be harmful.

A storage temperature of −70°C. is also satisfactory and equipment in which this temperature is maintained by mechanical refrigeration is now readily available. Still lower temperatures can be obtained in this way but the apparatus is apt to be rather expensive.

The ordinary commercial deep freeze operates at a much higher temperature, usually in the range −20° to −30°C. This is not satisfactory for storing artery grafts (p. 430). Bone grafts on the other hand are commonly stored at this temperature and give quite good results (p. 364), although it seems likely that storage at −70°C. or lower would be preferable.

The position with cartilage grafts is not altogether clear. Many surgeons have reported good results with grafts of dead cartilage (p. 387), including cartilage which had been stored frozen at about −20°C. without any attempt to maintain viability (Longmire, Cannon and Weber, 1954), but Gibson (1957) has stressed the advantages of living cartilage. He has attempted to preserve cartilage in a viable state by freezing after pre-treatment with glycerol, but so far without success. He has suggested that the reason for this failure is that the cartilage matrix prevents the glycerol from entering the chondrocytes.

FREEZE-DRYING

In freeze-drying* the tissue is frozen, and then kept frozen while it is subjected to a high vacuum; under these conditions ice is removed from the tissue by sublimation. A simple apparatus† for freeze-drying tissue is illustrated in Figure 19; a more elaborate one in Figure 50. The tissue is frozen usually either to −79°C. with a mixture of alcohol, acetone and solid carbon dioxide, or to −190°C. with liquid nitrogen, and then placed in the apparatus in the position shown. The vacuum may be produced either by a mechanical pump alone or by a diffusion pump backed by a suitable mechanical pump.‡ Water is collected and prevented from reaching the pump by a trap cooled with liquid air, liquid nitrogen, or a mixture of alcohol and solid carbon

*Sometimes called lyophilization.

†Centrifugal freeze-drying plants used for freeze-drying solutions and suspensions are not suitable for macroscopic pieces of tissue.

‡The degree of vacuum used by different investigators ranges from 6.10^{-2} to less than 1.10^{-6} mm. mercury.

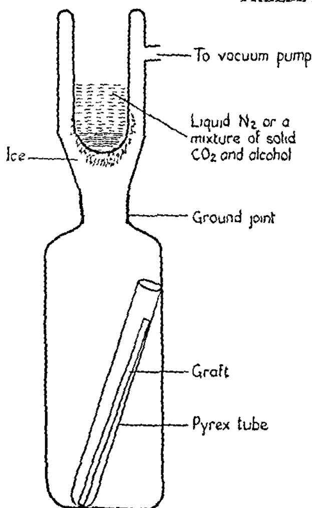


Fig 19 A simple apparatus for freeze drying artery grafts.

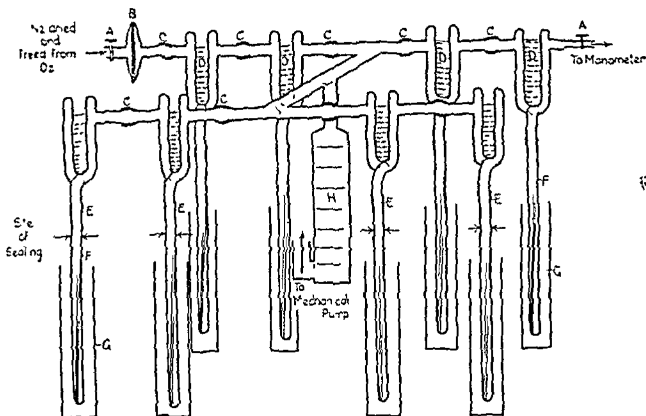


Fig 30 Apparatus for freeze-drying 8 artery grafts simultaneously A separate condenser is provided for each graft For the first four hours the tubes containing the grafts are surrounded by vacuum flasks containing solid CO_2 When drying is complete the *pumps are turned off* and dried oxygen free nitrogen is admitted *to bring the pressure in the system* to atmospheric pressure The tubes containing the arteries are then sealed with a hot flame at the sites indicated by the arrows The ground joint on the upper end *of each tube* is recovered and sealed on to a new tube for future use A Stop cock B Pipe line steri *lising* filter C Spherical ground joint D Condenser cooled with liquid nitrogen E Ground joint F Tube containing artery graft G Removable vacuum flask containing solid carbon dioxide and alcohol *H Diffusion pump*

dioxide The tube containing the tissue is usually cooled externally, *at least during the* initial *stage of* the process, but this is not always necessary because the heat loss repre senting the latent heat *of sublimation* may suffice to keep the tissue frozen If external cooling is applied care must be taken to avoid cooling the tube *containing* the tissue down to the temperature of the water trap, otherwise ice will collect in the wrong place If, as in the illustration, liquid air is used in the trap the tube containing the tissue may be *cooled with solid carbon dioxide* If, as is sometimes done, a solid carbon di oxide alcohol mixture *is used in the trap* the tube *containing the* tissue *should not be* cooled to the same extent

After being freeze dried the tissue may be sealed up immediately *in vacuo* or in an at- mosphere of nitrogen, alternatively it may be further dehydrated over phosphorus pentoxide before being sealed up

For immediate sealing two techniques are available The first method is to stop the pump, remove the tube containing the tis- sue from the apparatus, fit a rubber cap, *re evacuate the tube* (or fill it with nitro- gen) by means of a needle pushed through the cap, and finally seal, after withdrawing *the needle, by coating the cap* with molten vacuum wax (Eastcott, Holt, Peacock and *Rob,* 1951) The *second method is to seal the tube* before it is removed from the apparatus This is easy if the system has

been filled with nitrogen at atmospheric pressure. It is more difficult when the system is evacuated, but can be done, as Hufnagel, Rabil and Reed (1954) have shown, if the wall of the tube has been previously thickened at the site at which it is to be sealed.

Behaviour of Freeze-dried Grafts

The term freeze-dried graft will be used to denote a graft of tissue which has been freeze-dried and subsequently rehydrated by soaking in physiological saline or water

Some years ago Webster (1944a) reported one instance of partial survival of a human skin graft which had been "lyophilized" and then stored at room temperature for 17 days, the graft was, however, less satisfactory than a fresh graft Later, Gye and his colleagues (Gye, 1949, Gye, Begg, Mann and Craigie, 1949), after many unsuccessful attempts, succeeded in propagating a number of mouse tumours by means of freeze-dried tumour tissue They interpreted this as evidence that tumours may be propagated by a virus in the absence of living tumour cells, but later investigators (Passey and Dmochowski, 1950 Passey, Dmochowski, Lasnitzki and Millard, 1950) have shown by tissue culture that tumour tissue freeze-dried by methods similar to those used by Gye *et al* does contain some living cells.

In the experiments we have been considering the tissue was rehydrated and transplanted or explanted either immediately after freeze-drying or after a short period of storage. Attempts to use freeze-drying as a means of storing tissue in a viable state for long periods have been uniformly unsuccessful.

The reason for this failure is not far to seek. None of the investigators mentioned above made any attempt to determine the degree of dehydration to which the tissue was subjected. More recently, however, Billingham and Medawar (1952) have shown that rabbit skin, freeze-dried either immediately or after pre-treatment with glycerol, fails to survive* if the final overall water content is less than about 25 per cent; and these authors have suggested that for every mammalian tissue there is a limit beyond which dehydration causes cell death. On the other hand, tissue which is not dehydrated, or, it seems, is dehydrated to an extent insufficient to cause death, soon ceases to be viable when stored at room temperature.

Freeze-drying is, nevertheless, a useful method of storage in the special case of grafts of blood vessels and other tissues which need not remain viable.

Blood Vessels

Freeze-dried arterial grafts were first used by Marrangoni and Cecchini (1951), and have since been used extensively both in experimental animals and in man (Hyatt, Turner, Bassett, Pate and Sawyer, 1952; Pate, Sawyer, Deterling, Blunt and Parshley, 1953; Pate and Sawyer, 1953; Hufnagel, Rabil and Reed, 1954; Pate, 1954; Eastcott, Holt, Peacock and Rob, 1954; Rob, 1954; Creech, Cooley and DeBakey, 1954; Lehr, Blakemore, Sawyer, Glauser and Johnson, 1955; Sauvage, Wesolowski and Pinc, 1955) Freeze-dried arteries may be stored for long periods, and as far as is known indefinitely, at room temperature, either *in vacuo* or in an atmosphere of nitrogen. After being reconstituted they may be used successfully not only as autografts and homografts but also as heterografts (Hufnagel, Rabil and Reed, 1954). As expected, however, explants of freeze-dried arteries yield no growth in tissue culture (Marrangoni and Cecchini, 1951; Pate *et al*, 1953).

*Billingham and Medawar reconstituted the freeze-dried grafts and then transplanted them back to the original donors. They thus proved that, under the conditions stated, the skin failed to survive the combined operations of freeze-drying and reconstitution. It is conceivable that the crucial damage may occur during reconstitution

According to Pate and his colleagues (Pate *et al.*, 1953; Pate and Sawyer, 1953; Sawyer and Pate, 1953a, b) freeze-dried homografts become re-endothelialized sooner, and are less liable to become thrombosed, than fresh homografts. The superiority of freeze-dried grafts has been attributed to two factors: absence of intimal electrostatic charge, and diminished antigenicity. The first of these factors was investigated by Sawyer and Pate (1953a, b), who reported that the inner surface of a normal artery is negatively charged, that of a fresh homograft is positively charged, and that of a freeze-dried graft carries no charge. The positive intimal charge of fresh homografts is disadvantageous because it attracts red blood cells, which are negatively charged, and so predisposes to thrombosis.

A point of much interest and importance is the extent to which arterial grafts should be dried. Many authors appear to have assumed that the more water removed the better, but Sewell and Koth (1954) investigated the matter and concluded that grafts dried until the final water content was about 5 per cent were more satisfactory than those with a final water content of only 1 per cent.

In comparing different methods of storage the basic criterion is the long term behaviour of the grafts. It would clearly be advantageous to devise a test which would yield similar results in a shorter time. With this objective in mind, Gentsch, Waters and Glenn (1954) subjected aortic homografts which had been preserved by freeze-drying to stress by giving repeated intravenous infusions of an egg yolk saline mixture to the host, starting eight to ten weeks after grafting. They found that the grafts became infiltrated with fat, foam cells and inflammatory cells, and about half of them became calcified. The adjacent host aorta was much less affected. This test would seem to merit further investigation but at present the significance of the findings is not clear.

Bone

Homografts of freeze-dried cancellous bone have been studied by Kreuz, Hyatt, Turner and Bassett (1951), and by Turner (1952). They appear to stimulate the formation of host bone and have been reported to give excellent functional results (p. 365).

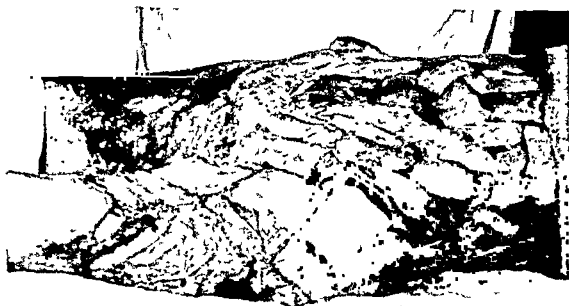


Fig. 51. Homografts of freeze-dried skin 4 days after grafting

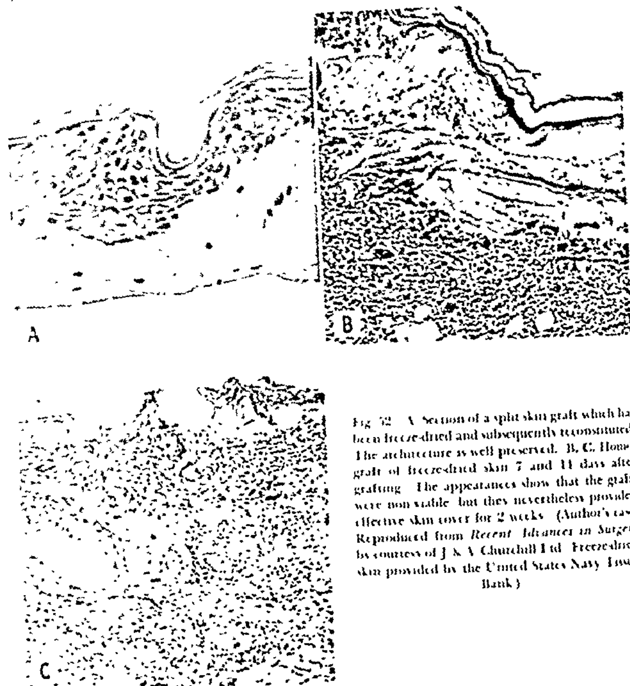


Fig. 52. A. Section of a split skin graft which has been freeze-dried and subsequently reconstituted. The architecture is well preserved. B, C. Homograft of freeze-dried skin 7 and 11 days after grafting. The appearances show that the grafts were non-viable, but they nevertheless provided effective skin cover for 2 weeks. (Author's case. Reproduced from *Recent Advances in Surgery* by courtesy of J. & A. Churchill Ltd. Freeze-dried skin provided by the United States Navy Tissue Bank.)

Other Tissues

Weiss [illegible] and Weiss and Taylor (1943 [illegible]) [illegible] in cats and monkeys that [illegible] homografts of reconstituted freeze-dried nerve evoked less lymphocytic reaction than fresh homografts. Grafts up to 3 cm. long became re-innervated, and functional recovery followed. More recently Sanders (1954a, b) has studied the behaviour of longer (5 cm.) grafts in rabbits. He found that freeze-dried and fresh homografts did not differ with respect to the time of onset, or the degree, of functional recovery they yielded, though he confirmed Weiss' observation that there is less lymphocytic reaction in the early stage with freeze-dried grafts.

Strong and his colleagues (Strong, Turner and Bassett, 1953; Strong, 1954) used freeze-dried human skin clinically as a dressing, and reported that grafts of this material behaved for a time like fresh grafts, becoming

firmly attached for about the same time and later sloughing Buchan and Woodruff (unpublished) confirmed this as far as the macroscopic appearance of the grafts was concerned (Fig 51) They found however, as would be expected, that although the tissue appeared histologically normal when rehydrated (Fig 52A) it was obviously dead as judged by its histological appearance a day or two after grafting (Fig 52B, C).

Katzin (1947) has reported unfavourably on reconstituted freeze dried corneal grafts Nine such grafts in human patients, though clear at the time of transplantation, all became opaque within a period of three weeks

FIXATION BY CHEMICAL AGENTS AND HEAT

Grafts which are not required to be viable may be preserved by fixation and storage in chemicals such as formalin, alcohol and merthiolate, or by boiling or autoclaving These methods have the advantage that bacterial contamination is easily prevented, and even contaminated grafts may be sterilized On the other hand they cause gross alterations in protein structure, and grafts so altered are sometimes, though not always, less satisfactory than those preserved in other ways

Blood Vessels

Carrel (1908a) and Guthrie (1908b) showed that segments of aorta preserved in formalin could be used successfully as transplants in dogs, and this has been confirmed in a small number of experiments by Brunnen (1953) Carrel failed with transplants of smaller arteries preserved in the same way, but Nageotte and Sencert (1918d, 1919) obtained good results with grafts of dog carotid which had been preserved in alcohol

Formalin preserved arterial transplants have also been used clinically with moderately good results by Peirce, Rheinlander, Moritz, Gross and Merrill (1949), and van Weel* (1953), and Kimoto (1951) has reported favourably on arterial homotransplants and heterotransplants preserved in alcohol, but most investigators today hold that other methods of preservation, especially freeze-drying, give better and more uniform results

*Brunnen and van Weel both used 4 per cent formalin buffered to pH 5.6.

Cartilage

Cartilage preserved in alcohol (50 95 per cent) has been used in experimental animals (Prudden, 1881, Nageotte, 1922, Polettini, 1922) and also in man (Peer, 1938, 1939b). Transplants of this material, whether homologous or autologous, provoke more reaction than similar transplants of fresh cartilage, they may persist for a year or two, but are as a rule eventually absorbed

Cartilage preserved in merthiolate* (O Connor, 1939) is more satisfactory, and good results have been reported with both autotransplants and homotransplants of this material in man (Pierce and O Connor, 1938, O Connor and Pierce, 1938, Mowlem, 1941, Straith and Slaughter, 1941, Brown and De Mere, 1948), but cartilage preserved in this way appears to be less resistant to infection than fresh cartilage, and may be absorbed even in the absence of infection (p 387).

Heterotransplants of merthiolate-preserved ox cartilage have also been used clinically (p. 387). The earlier reports (Wardill and Swinney, 1947, Gillies and Kristensen, 1951) were very favourable but more recently it has been found that such transplants are usually absorbed within two years (Gibson and Davis, 1953)

Bone

Bone for grafting has been stored in alcohol (Christophe, 1923), ether (Calve, 1935)

* The usual solution consists of 1 part of merthiolate in 1000 of saline

and merthiolate (Reynolds and Oliver, 1949; Harmon, 1950; Gordon and Welsh, 1951; Nisbet, unpublished).

Gallie (1918) studied the behaviour of grafts of boiled bone, and Orell (1937) prepared what he called *os purum*, consisting of bone freed of fat, connective tissue and protein, by a lengthy physico-chemical process which included soaking in warm potassium hydroxide.

The behaviour of grafts of these materials is described in Chapter 18.

Cornea

Maumenee and Kornblueth (1948), in a study of various methods of storing rabbit cornea, found that preservation in formalin was unsatisfactory. Out of fifteen grafts stored in this way only one remained even partially clear after grafting.

STERILIZATION OF TRANSPLANTS

To avoid bacterial contamination transplants must be removed from the donor either during life or soon after death, and a scrupulous aseptic technique must be employed. It is often inconvenient and sometimes impossible to fulfil these conditions, especially with human material, and the problem of sterilization then arises.

The methods of preservation by chemicals and by heat considered in the last section provide means of sterilization of varying degrees of efficacy, but have only a limited application.

Several other methods have been used for sterilizing blood vessel grafts including immersion in formalin (Brunnen, 1953; Ross, 1954a) or liquid ethylene oxide (Hufnagel, Rabil and Reed, 1954) for a short time followed by washing or freeze-drying; exposure to gaseous ethylene oxide (Hufnagel *et al.*, 1954; Hufnagel, 1954) or beta-propiolactone (Szilagyi, Overhulse, Shonnard and LoGrippo, 1954; LoGrippo, Overhulse, Szilagyi and Hartman, 1955) after freeze-drying; treatment with antibiotics (Rob, personal communication); and irradiation (Meeker and Gross, 1951a, b), Hui, Keefer, Deterling, Parshley, Humphreys and Glenn, 1951; Brunnen, 1953). The techniques used, and the results obtained, will be discussed in Chapter 21.

Gamma radiation from radioactive cobalt has also been used experimentally for sterilizing cartilage, and Lynch, Asbury and Dingman (1956) found that in dogs homografts sterilized in this way survived longer than homografts of fresh cartilage.

CHAPTER 10

Transplantation as an Instrument of Research
I. Genetics, Experimental Embryology, Physiology

The scientific worker may be interested in transplantation for two main reasons. On the one hand he may regard the search for general laws governing the behaviour of transplants as an end in itself. On the other hand he may be interested in transplantation only as a means to an end, that is to say as an instrument of research in some other field. In this chapter and the next we shall discuss transplantation from the latter point of view, and consider some examples of its application to research in Genetics, Experimental Embryology, Physiology, and Experimental Pathology.

GENETICS AND EXPERIMENTAL EMBRYOLOGY

Transplantation has many applications in the related fields of genetics and experimental embryology. In some instances transplantation has led directly to the solution of a problem; in others it has revealed a new problem, or a new aspect of an old problem, of fundamental importance. We shall consider four examples:

1. The use of skin grafting to distinguish between monozygotic and dizygotic twins.
2. The study of factors influencing differentiation during embryonic development.
3. The immunological problem of pregnancy in viviparous animals.
4. The study of the influence of somatic changes on inherited characteristics.

The Recognition of Monozygotic Twins

Tests based on the behaviour of skin homografts may be of great value when it is required to determine whether given twins are monozygotic or dizygotic. The simplest test is to exchange grafts between the animals in question. Provided that the technique is satisfactory, failure of either graft to survive permanently proves that the twins are dizygotic. The converse, however, is not always true. In cattle, as we saw in Chapter 7, dizygotic twins are often genetic chimeras, in which event homografts exchanged between them may survive permanently, and the same phenomenon occurs occasionally in other species.

Another test is therefore needed, and one which seems likely to be valuable has been suggested by Anderson, Billingham, Lampkin and Medawar (1951). In this test skin grafts are made simultaneously from the two animals in question to a third animal of the same species which is unrelated to either. The grafts may be expected to survive for the same length of time, and to evoke the same degree of histological reaction, if, and only if, the twins possess the same, or very nearly the same, histocompatibility genes (p. 60), and if the parents are

genetically diverse this is exceedingly unlikely to happen in most species* unless the twins are monozygotic. If a very slight difference in survival time is observed, and its significance is uncertain, a refinement of the test may be used in which a fourth animal is first immunized by a graft from one of the twins and later receives simultaneous grafts from both.

A similar but in one way more difficult problem arises when it is required to determine whether animals of an inbred strain are nearly homozygous. Here the most useful test is to exchange skin grafts between randomly selected pairs of animals of the same sex.† If most of the grafts are rapidly destroyed it is apparent that the strain is far from uniform. If, on the other hand, all, or nearly all of the grafts survive permanently it follows that there is a high degree of genetic uniformity *as far as the histocompatibility genes are concerned.* This qualification, which could for practical purposes be omitted in the previous problem, is now essential because after some generations of in-breeding there may well be a high degree of uniformity in respect of some genes but considerable diversity in respect of others.

Experimental Embryology of Vertebrates

Experimental embryology is based to a large extent on four techniques: *extirpation* or isolation of parts of embryos, *transplantation, explantation* and *vital staining.*

Transplantation has proved to be of special importance in the study of determination and self-differentiation, and embryonic induction. Much of the experimental work on vertebrate development has been carried out with amphibian and avian embryos; in this section, therefore, we shall extend our terms of reference to include consideration of transplantation in non-mammalian vertebrates as well as in mammals.

It will be convenient to explain briefly the meaning of some technical terms, but for a full discussion the reader should consult one of the standard authorities on experimental embryology (e.g. Huxley and DeBeer, 1934; Needham, 1936a, b, 1942; Spemann, 1938; Weiss, 1939; Hamburger, 1942).

In so far as a part will continue to develop in the same way under all conditions in which it is capable of developing at all, it is said to be *determined,* and to develop by *self-differentiation;* whereas in so far as its development is dependent on, and conditioned by, extrinsic factors the part is said to undergo *dependent differentiation.* The terms *self-differentiation* and *dependent differentiation,* which were introduced many years ago by Roux (1885), are convenient but it must be emphasized that they have only a relative significance; strictly speaking all differentiation is to some extent dependent on environmental factors.

Embryonic induction* may be defined as a process in which differentiation in embryonic tissue in a particular direction is dependent on and conditioned by contiguity with another tissue which is then said to function as an *inductor.* Tissue which is capable of being influenced by a particular inductor is said to be *competent* (Waddington, 1932) as far as that inductor is concerned. Some authorities use the term *organizer* as a synonym for inductor; others, however, use it in a more restricted sense which will be explained later (p. 187).

Determination and Self-Differentiation

In studying determination and self-differentiation the following questions, propounded by Hamburger (1942), are pertinent:

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*The hamster, as we have seen (p. 63), is an exception.

†Animals of the same sex are used to avoid the Eichwald Silmser effect (p. 59).

*As noted previously (p. 33) the term *induction* may be defined in a wider sense to include instances in adult organisms in which the growth of one tissue is influenced by contiguity with another.

1. At what stage of development does a given embryonic primordium become relatively self-differentiating?

2. What particular developmental processes or structural details of the primordium are self-differentiating at a given stage?

3. With respect to what extrinsic structures or factors is the primordium independent or dependent?

The two standard methods of dealing with these questions experimentally are, firstly *heterotopic transplantation*, which is usually homologous but sometimes autologous or heterologous,* and secondly *explantation*, of the primordium under consideration.

Transplantation has been used extensively in studying the capacity for self-differentiation of many embryonic structures.

The experiments performed in amphibians include *inter alia* homologous and heterologous transplantation of various regions of blastulas and early gastrulas to the coelomic cavity (Holtfreter, 1925, 1929) or eye socket (Durken 1925, Brautzmann, 1929 Kusche 1929) of older embryos † and autologous homologous and heterologous transplantation of limb primordia (Harrison, 1907 1918, 1921a Mangold 1929 Swett, 1937) balancer primordia (Harrison 1924) gill primordia (Harrison 1921b Severinghaus 1930 Rotmann 1935) and other structures to a variety of sites using donors at various stages of development and hosts of about the same stage as the donor in each case.

In chicks limb primordia (Murray and Huxley, 1925, Murray, 1926, Hunt, 1932, Hamburger 1938 1939, Hamburger and Waugh, 1940) and various organ primordia (Rienhoff, 1922 Danchakoff 1924, Hoadley 1924 1925, 1926, 1929 Willier, 1930, Rudnick, 1932, 1933, Jos 1939) have been transplanted homologously to the chorio-allantoic membrane the coelomic cavity, and the body wall of the embryo. In addition Willier and Rawles have investigated the chronology of melanophore migration (Willier and Rawles 1940 Willier 1941, 1942) by making homotransplants of epidermal structures or neural crest from embryos of pigmented fowls in various stages of development to White Leghorn embryos, and Rawles (1940) has studied the same phenomenon in mice by means of heterotransplants from mouse embryos to chick embryos.

Observations have also been made on the differentiation of various mammalian (mainly rat and mouse) embryonic tissues transplanted homologously to the brain of an immature but non-embryo recipient (Willis 1936 Tansley, 1946) or heterologously to the chorio-allantoic membrane (Nicholas and Rudnick 1933) *or the coelomic cavity* (Rawles 1940 Gluecksohn-Schoenheimer 1941) of the chick embryo.

Embryonic Induction

Embryonic induction has been studied in two main ways by extirpation and by transplantation. If after extirpation of an embryonic area an adjacent area fails to differentiate normally it is reasonable to postulate that the area removed is an inductor but crucial evidence can be obtained only by bringing the supposed inductor into contact with appropriate but as yet undetermined tissue from some remote part of the embryo. This may be achieved (a) by transplanting the supposed inductor itself, (b) by transplanting appropriate tissue to the vicinity of the supposed inductor, or (c) by

* The terminology used elsewhere in the book has been adhered to here, although experimental embryologists at times used the term *heterotransplantation* to denote transplantation between closely related species and *xenoplastic transplantation* to denote transplantation between individuals belonging to different genera, families or more distant taxonomic categories (cf. Hamburger 194[illegible]).

† Transplants to the coelomic cavity and eye socket usually remain freely suspended in the fluid in the cavity without becoming attached to the host, and on this account some experimental embryologists refer to them as explants. We shall, however, adhere to the terminology used elsewhere in the book.

transplanting, or explanting, the supposed inductor and the appropriate tissue to some neutral environment.

It will suffice to consider briefly two well known and much studied instances of embryonic induction: formation of the lens of the amphibian eye from epidermis under the influence of the eye cup, and the complex inductive effect of the primary organizer in the dorsal lip of the blastopore of amphibian gastrulas.

Lens Induction. The suggestion that the development of the vertebrate lens depends in some way on the optic cup was put forward many years ago by Herbst (1901) and others. Extirpation experiments (for review see Spemann, 1938) supported this conjecture in several, though not in all, species of amphibia, but the decisive proof came when Lewis (1904, 1907) showed in two species of frog (*Rana sylvatica* and *Rana palustris*) that the optic cup is capable of inducing a lens in epidermis which does not normally give rise to a lens at all. In one experiment he transplanted the optic cup autologously to a position beneath the epidermis of the trunk, and in another he transplanted epidermis from the trunk to a position overlying the optic cup, in both experiments a lens usually developed.

In *Rana esculenta*, on the other hand, Spemann (1912) found that a lens could not be formed from the epidermis of the trunk, or from the epidermis of the head apart from the presumptive lens-forming epidermis. This suggested either that the potency for lens formation is restricted, at the stage of development at which the experiments were performed,* to the presumptive lens-forming cells, or alternatively, that the power of the eye cup to induce a lens has been lost. To distinguish between these possibilities Filatow (1925) and Pasquini (1931) transplanted trunk epidermis heterologously from a species with more extended lens potency (*Bufo vulgaris*) to a site overlying the optic cup in *Rana esculenta.* The inductive capacity of the optic cup was shown by the fact that a lens formed in this epidermis. Since it can be shown by extirpation of the eye-rudiment that the lens is in fact determined in *Rana esculenta* at a still earlier stage of development it would seem that we have here an example of what Spemann (1938) calls *double assurance:* the lens can develop independently of the eye cup, but at the same time the cup retains its inductive capacity.

More recently Holtfreter (1934a, b) has shown that lens formation may be induced by transplants of a variety of tissues both living and dead, including boiled medullary plate, alcohol-killed endoderm, heterologous fresh liver and heterologous dead heart. These rather bizarre findings lend strong support to the view that lens-induction is a chemical phenomenon; at the same time they appear to the author to make it difficult to ascribe any teleological significance to the phenomenon of double assurance.

The Primary Organizer. In the course of experiments designed to study the degree of determination in various regions of the early urodele gastrula Spemann (1918) found that, if tissue from the neighbourhood of the dorsal lip of the blastopore of an early gastrula was transplanted homologously to the flank of another gastrula, a secondary embryo with neural tube, notochord and somites was formed at the site of transplantation.

At the time Spemann believed that the secondary embryo was formed wholly from the transplant but this view proved to be erroneous. The true state of affairs was discovered by transplanting tissue from the region of the dorsal lip of the blastopore of *Triton cristatus* heterologously to the flank or ventral region of a gastrula of *Triton taeniatus* or *Triton alpestris* of ap-

*The late tail bud stage

proximately the same age (Spemann and Mangold, 1924). Donor and host tissue could then be readily distinguished owing to a difference in pigmentation, and the contribution of each to the secondary embryo ascertained. It was found that mesodermal structures, such as notochord and somites, developed either from the transplant alone by self-differentiation, or partly from the transplant by self-differentiation and partly from the host tissue by induction; while the other tissues of the secondary embryo developed wholly from the host tissue.

The tissue of the dorsal lip of the blastopore is thus able to self-differentiate, and at the same time to transform the tissues of the host by induction, and assimilate them, or be assimilated by them, to form an integrated whole. In view of this remarkable property the name *organizer* was given to it by Spemann and Mangold. The term is now also applied to other inductors which operate later in development, and the tissue of the dorsal lip is therefore referred to as the *primary organizer*.

It would lead us too far afield to consider the further analysis of organizer activity and its role in normal development, but a full discussion will be found in Spemann's (1938) book.

Influence of Maternal Environment on Offspring

It is becoming apparent that some characteristics which were assumed to be genetically determined may be altered by changing the maternal environment during development.

One way of investigating this is to transplant a fertilized ovum to a female of a foreign strain. The transplant possesses genes absent from the host, but the normal embryo also possesses genes absent from its own mother (except in the special case of inbred homozygous strains) and, as we shall see later (p. 188), there are various mechanisms which protect the developing embryo against the immunological hazards of pregnancy. Ovum transfer has been used in mice by McLaren and Michie (1958), and several other investigators, and they have shown that the number of lumbar vertebrae in the offspring may be altered as a result of the transplantation.

An alternative procedure is to transplant ovarian tissue orthotopically to an F1 hybrid which has been oophorectomized or whose own ovaries have been destroyed by irradiation as described by Parrott and Parkes (Parrott and Parkes, 1956a, b; Parrott, 1958b), and then mate this animal with a male of the donor strain or of some other suitable strain.

Orthotopic ovarian transplantation following surgical oophorectomy has been used by Guthrie (1907b, 1911), Castle and Phillips (1909, 1913), Robertson (1940, 1942, 1945), and Russell and his colleagues (Russell and Douglass, 1945, 1946; Russell and Hurst, 1945; Russell and Gover, 1950), and their work is reviewed in Chapter 21. The method is of limited application because it is technically difficult to remove the host ovaries completely and at the same time to achieve a fully effective graft.

Parrott's procedure gives excellent results in mice. It can also be used in a modified form in rats and hamsters (Parrott, 1958a), which are too sensitive to irradiation effects to be sterilized by whole body irradiation, if appropriate methods of shielding are used. It does not yet appear to have been tried in other species.

The use of Fl hybrids of the donor and another strain as recipients imposes a serious restriction. An alternative is to use as recipients animals made specifically tolerant of donor strain tissue (Chapter 7).

The Immunological Problem of Pregnancy

The question now to be considered may be stated as follows. how does the foetus of a viviparous animal manage to sur-

vive and develop throughout the period of gestation when its tissues are antigenically different from those of the mother? Why does it not suffer the usual fate of homotransplants?

It is, of course, well known that human foetal red cell antigens, especially the D antigen of the Rh series, may evoke a state of immunity in the mother which results in foetal disease or death, but they do so much less often than one would expect from a knowledge of the immunizing effect of transfusions of Rh incompatible blood (*see* Race and Sanger, 1958) and so far there is no evidence that the tissue antigens responsible for the reaction to ordinary homografts play a similar role. It may well be that some unexplained foetal deaths are due to immunization by tissue antigens, for the dearth of *in vitro* tests for the corresponding antibodies has made it difficult to obtain decisive evidence: the fact remains, however, that pregnancy commonly runs its course without mishap in spite of genetic—and hence potentially at least, antigenic—differences between foetus and mother.

Medawar (1953) has suggested three factors which may account for this apparent anomaly: the anatomical separation of the foetus from the mother, the antigenic immaturity of the foetus, and the immunological inertness of the mother. A further possibility which merits consideration is that the foetus possesses a defence mechanism which enables it to survive in an immunologically unfavourable environment.

There is no reason to suppose that immunological inertness of the mother is of decisive importance. Admittedly in rabbits, as Heslop, Krohn and Sparrow (1954) have shown, skin homografts transplanted to pregnant does between the 20th and 24th day of gestation survive for about twice as long as homografts transplanted to males or non-pregnant females, and it seems likely, as Heslop *et al.* have suggested, that this is due to increased secretion of corticosteroids by the pregnant animals. Homografts transplanted to rabbits earlier or later in pregnancy survive only for the normal time however, as do homografts in pregnant mice (Medawar and Sparrow, 1956).

Less is known of the antigenic status of the foetus in respect of the nuclear antigens responsible for transplantation immunity, but Billingham, Brent and Medawar (1956b) refer briefly to experiments which show that in mice these antigens do in fact mature before the end of intra-uterine life.

The anatomical separation of foetus and mother resembles in some ways the separation between graft and host in diffusion chamber experiments (p. 114), but with the important difference that the foetus is well nourished. It is generally accepted that cells do not normally pass from the maternal to the foetal circulation or *vice versa*, but are occasionally able to do so owing to the development of leaks. The frequency with which such leaks develop in different species is unknown but several cases have been reported in which erythrocytes of foetal origin have been demonstrated in the blood of women during pregnancy or in the puerperium (Chown, 1954; Dunsford, 1957; Goodall, Graham, Miller and Cameron, 1958; Weiner, Child, Garvie and Peek, 1958). On the other hand in mammals humoral antibodies may be transferred from the mother to the young *before birth*, as in man, the guinea pig and the rabbit; *after birth*, via the colostrum and milk, as in horses, pigs and cattle; or *partly before but mainly after birth*, as in dogs, rats and mice (Brambell, Hemmings and Henderson, 1951). The extent to which transfer occurs before birth depends partly on the structure of the barrier between the maternal and foetal circulations in the placenta, and partly on the availability of extra-placental routes for the interchange of substances between the two circulations.*

**Placenta* here means the mammalian chorio allantoic →

It might be expected therefore that the separation of foetus and mother would prove to be the decisive factor in protecting the foetus against the immunological hazards of pregnancy but until recently direct experimental evidence was lacking.

The author (Woodruff 1957f) has now investigated the matter in both rats and rabbits.

In a preliminary experiment mature female Wistar rats each received a massive skin graft from a male hooded donor. Four to six weeks later each female was mated with the hooded male from which it had received a skin graft. It was found that pregnancy occurred and ran its normal course and that the mean size of the litters was the same in these rats as in non-grafted Wistar females mated with hooded males. The experiment was repeated in rabbits using does and bucks drawn from a mixed population and yielded similar results. These findings were not unexpected because Mitchison (1953) had already reported that when CBA female mice were mated with male (CBA × A) F1 hybrids it made no significant difference to the average size of the litter or the distribution of the various genotypes among the offspring whether the females had previously been immunized with erythrocytes or tumour homografts from mice of strain A or not, and while the experiments were in progress Medawar and Sparrow (1956) found that CBA female mice which had been immunized with homografts of A strain skin became pregnant when mated with A strain males; but confirmation was needed before proceeding further.

[illegible] that the first of these [illegible] was the [illegible] has been [illegible] by Brambell [illegible] that [illegible] of the placental barrier [illegible] transfer of [illegible] late stages of pregnancy takes place [illegible] the uterine lumen and [illegible] the yolk-sac placenta [illegible] Brambell, Hemmings and [illegible] the path [illegible] to the foetus [illegible] yet been established.

In the experiment proper female Wistars which had each received a skin graft from a hooded male four to six weeks previously were mated as before with the skin donor and on the 15th day of pregnancy one embryo was removed and the two hindquarters were grafted to the mother, one being placed in each flank beneath the panniculus carnosus muscle. The same manoeuvre was performed with control (non-grafted) Wistars which had been mated with hooded males. One graft was removed for examination after being *in situ* for six days (i.e. just before parturition occurred) and the other eight days later.

Twelve of the eighteen immunized animals and thirteen of the twenty-two controls became pregnant. The subsequent operative interference was well tolerated and all except two of the animals which became pregnant littered 22 to 27 days (mean 23 days) after mating, i.e. 1 to 6 days after removal of the left graft and before removal of the right graft.

The young appeared healthy and developed normally for 4 weeks after which they were destroyed. Most of them (72 per cent) showed areas of black pigmentation. There was no significant difference in the average number per litter from immunized and non-immunized mothers.

The histological findings (Figs 53–54) differed greatly in the two groups.

In the control animals grafts removed after 6 days all contained healthy cartilage and stratified squamous epithelium together with much undifferentiated mesenchymal tissue. In addition bone, muscle and metanephric tubules were sometimes present. There were numerous mitotic figures especially in the cartilage and squamous epithelium. *There was no evidence of host reaction.* Grafts removed after 14 days were well vascularized and contained abundant cartilage, bone and bone marrow, cysts lined by stratified squamous epithelium, hair follicles, and occasionally meta


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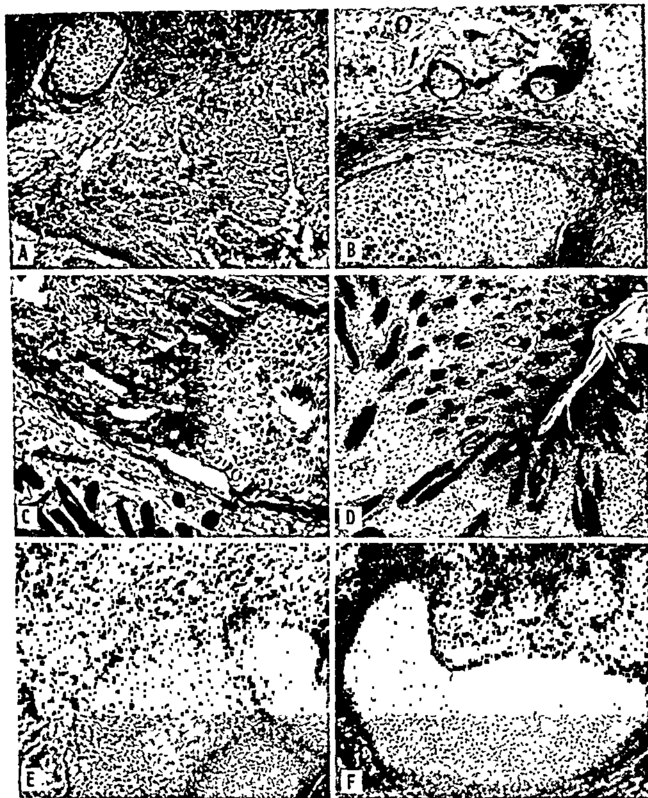


Fig. 51. Photomicrographs of grafts from foetus to mother in immunized rats, and in control and immunized rabbits. A. Six day graft in immunized rat showing areas of necrosis and a small island of cartilage. B. Fourteen day graft in immunized rat showing somewhat degenerate looking cartilage and mature connective tissue. C. Fourteen day graft in control rabbit showing cartilage, developing bone and hair follicles. D. Fourteen day graft in control rabbit showing a cyst lined by stratified squamous epithelium and numerous hair follicles. E. Six day graft in immunized rabbit showing areas of necrosis and two small islands of cartilage. F. Fourteen day graft in immunized rabbit. All that remains is a solitary island of cartilage. Haematoxylin and eosin. × 72. (Reproduced from *Proceedings of the Royal Society* by courtesy of the Royal Society.)

nephric tubules. There were numerous mitotic figures, especially in the cartilage and squamous epithelium. Some grafts showed little or no evidence of host reaction, but in others there was round cell infiltration resembling that seen in homografts of adult tissue in non-immunized animals.

In the immunized animals five of the twelve grafts removed after 6 days contained no recognizable foetal tissue, and in four others the only recognizable foetal tissue was cartilage. The remaining three grafts contained cartilage and cysts lined by degenerate stratified squamous epithelium. No mitotic figures were found. There were areas of necrosis and haemorrhage, surrounded by young connective tissue containing numerous engorged blood vessels packed with red blood cells and dead leucocytes. Many of these vessels showed erosion of the endothelium. The histological picture thus closely resembled that seen in grafts of adult tissue in previously immunized animals. At 14 days the grafts had disappeared completely in three animals, but in the other nine animals there were scattered islets of cartilage surrounded by fairly mature connective tissue which sometimes contained accumulations of round cells. None of the grafts contained stratified squamous epithelium but one showed metanephric tubules. No mitotic figures could be found.

A similar procedure was carried out in rabbits using laboratory bred albino females and wild grey males. Two females were mated with a single male from which one of the females had received four large pinch skin grafts (p. 7) four weeks previously. Sixteen days after mating one embryo was removed from each female which had become pregnant, and the two hind limbs were dissected free and grafted intramuscularly to the mother. The left graft, with adjacent host tissue, was removed for histological examination after 6 days and the right graft after 14 days.

Six of the nine immunized animals and six of the nine non-immunized controls became pregnant. The subsequent operative interference was again well tolerated and all the animals which became pregnant littered 31 to 33 days after mating, i.e. 1 to 3 days after the second biopsy. With the exception of two litters (both from control animals), the members of which were born dead or died soon after birth, the young appeared healthy and developed normally. The number per litter ranged from one to five. The average was slightly greater from the control animals, but the difference was clearly not significant.

The histological findings (Fig. 54) closely resembled those in the rats.

In the control animals grafts removed after 6 days contained healthy cartilage, cysts lined by actively proliferating stratified epithelium, and sometimes foetal muscle, together with much undifferentiated mesenchymal tissue. There were numerous mitotic figures. There was no evidence of host reaction. Grafts removed after 14 days were well vascularized and contained abundant cartilage, bone and bone marrow, cysts lined by stratified squamous epithelium, and hair follicles. Mitotic figures were present but appeared to be less numerous than in grafts removed after 6 days. There was considerable round cell infiltration.

In the immunized animals two of the grafts removed after 6 days contained cartilage but no other recognizable foetal tissue; the other grafts contained in addition cystic spaces lined by degenerate but recognizable stratified epithelium. There were numerous areas of necrosis, surrounded by young connective tissue containing engorged blood vessels packed with red blood cells and dead leucocytes. At 14 days the grafts had disappeared in two animals and all that remained in their place was a small scar. In the other animals there were scattered areas of cartilage surrounded by fairly mature

connective tissue containing accumulations of round cells.

The behaviour of the foetal tissue grafts in the control animals in both experiments provides strong evidence that, in both rats and rabbits, the mother does not become effectively immunized against foetal tissues during normal pregnancy. The observation that foetal tissue grafted intramuscularly to the mother on the 15th or 16th day of pregnancy evokes a state of immunity which is sufficient to result in characteristic histological changes in grafts examined 11 days later would appear to indicate, however, that foetal tissue is antigenically competent and that the mother is capable of immunological response. Moreover these properties must be present before the immunity is manifest, and hence—at any rate in rabbits*—before the end of the normal gestation period.

It may well be that the foetal tissues are to some extent antigenically immature, or that the mother is to some extent immunologically inert, or both; indeed this is suggested by the fact that grafts from foetus to mother were only beginning to show evidence of host reaction at a stage when homografts of adult skin to non-pregnant hosts would have been completely destroyed. It is clear, however, that any such antigenic immaturity and immunological inertness are far from absolute, and it appears unlikely that these factors by themselves would suffice to overcome the immunological hazards of pregnancy.

The behaviour of the foetal tissue grafts in the immunized animals shows that females which receive a skin graft from the male with which they are subsequently mated are effectively immunized against grafts from foetuses resulting from the union. This is not surprising since the foetus derives from the father many genes foreign to the mother and is in consequence likely to possess, at least after a certain stage of development, tissue antigens not possessed by the mother. It is significant, however, that, even when a high degree of immunity to foetal tissues is produced experimentally in this way, conception occurs as readily as in non-immunized animals and pregnancy runs its normal course. It has already been argued that foetal antigenic immaturity and maternal immunological inertness are not sufficient to overcome the immunological hazards of pregnancy. This further observation—which holds good in both rats and rabbits—would seem to imply that these factors are, in addition, not necessary, and that the decisive factor—which is both necessary and sufficient—is the anatomical separation of mother and foetus. The only alternative explanation which has not been entirely excluded but appears to be less likely, is that this separation is sufficient but not necessary on account of some unspecified defence mechanism which enables an intact embryo, in contra-distinction to a free graft of part of an embryo, to survive in an immunologically unfavourable environment.

*The same [illegible] may [illegible] be true of rats. [illegible] be [illegible] because [illegible] the first experiment [illegible]

The Influence of Somatic Changes on Inherited Characteristics

Many experiments have been designed to test the hypothesis that a somatic change brought about by environmental factors may in the course of generations become hereditarily fixed and inherited independently of the environmental stimulus (*see* Waddington 1939; Medawar 1957). The available evidence offers no compelling reason for accepting the hypothesis, but certainly does not exclude it.

Various investigators, including Castle and Phillips (1903, 1913) and Guthrie (1907, 1911), have tried to attack the problem by ovarian transplantation, and their

work is reviewed in Chapter 21. Unfortunately a severe, and at the time unexpected, limitation was imposed by the homograft reaction, but it should now be possible to overcome this by using animals made specifically tolerant by injecting them before they were immunologically mature with donor strain cells (Chapter 7).

Another procedure which might prove illuminating would be to study the inheritance of various characters by the offspring of artificial chimeras produced by homotransplantation of appropriate cells either before the development of immunological maturity or after total body irradiation. If irradiation were used steps would have to be taken to protect the animals' gonads. This could be done by shielding or by removing the gonads temporarily and replacing them orthotopically after the irradiation, but it would have to be determined whether these procedures prevented the development of chimerism.

The lymphocyte would seem likely to be the most appropriate cell to use in experiments of this kind if current notions concerning its role as a vector of nucleoprotein turn out to be correct. Following irradiation however other cells would probably* have to be transplanted in addition to keep the animal alive.

*The possibility of repopulating the haemopoietic tissues of lethally irradiated animals by transplanting thoracic duct lymphocytes alone is being investigated in the author's laboratory. Present indications are that this procedure may prolong survival sufficiently to permit regeneration of host haemopoietic tissue after irradiation only slightly in excess of the minimum lethal dose, but no evidence has yet been obtained to suggest that the transplanted lymphocytes can differentiate into other haemopoietic cells.

PHYSIOLOGY

Transplantation provides a useful means of studying the effect of denervation and other environmental changes on the function of organs and tissues. The procedures used include transplantation of viscera by vascular anastomosis, pedicle transplantation of viscera with or without subsequent division of the pedicle, and free transplantation of tissue.

Autotransplants are commonly employed, but it might be advantageous sometimes to use either an isotransplant, or a homotransplant to a recipient made specifically tolerant of donor tissue by the procedures described in Chapter 7.

Effects of Denervation on Viscera

Attempts to denervate a viscus by direct attack on autonomic nerves and nerve-filaments is apt to yield disappointing results, but complete denervation can always be achieved by excising the viscus and then replacing it in its original site as a transplant by vascular anastomosis.

Renal transplants have been widely used in acute experiments by Brull and his colleagues for studying the metabolism of the denervated kidney (Dor and Brull, 1910), and the effect of changes in arterial blood pressure on renal blood flow and urine secretion (Brull and Louis-Bar, 1950, 1953a).

In most of this work both kidneys were transplanted with segments of aorta and vena cava to the neck, usually of another dog, by the technique originally described by Brull in 1931. When necessary the blood pressure in the renal artery was controlled by incorporating on the arterial side of the system a form of roller pump devised by Brull (1950) and termed by him a mechanical heart.* The effect of denervation has

*With this apparatus the blood is "squeezed" not in a piece of rubber tubing but in a segment of fresh aorta connected to the renal artery of the transplant and the carotid artery of the recipient by Payr cannulae. It can therefore be used with coagulable blood.

been studied by comparing the findings with those of experiments in which inervated kidneys were perfused *in situ* by the technique of Shipley and Study (1951) or Brull and Louis-Bar (1953b).

Long term studies of transplanted kidneys have been undertaken by many observers (Chapter 25), and a beginning has been made with the study of the transplanted lung and heart (Chapter 26).

In interpreting the results of experiments of this kind it must be remembered that transplantation and replacement of a viscus does more than interrupt the nerve supply. It subjects the viscus to temporary deprivation of a blood supply, interferes with its normal lymphatic drainage, and often permanently interrupts subsidiary vascular connexions, as for example the bronchial vessels which are ligated when the lung is transplanted. In addition, in the special case of the kidney, it involves division and anastomosis of the ureter. Nevertheless, in spite of these complicating factors, the method may yield much valuable information which could not easily be obtained in any other way.

When transplantation by vascular anastomosis is not feasible owing to the vessels being too small pedicle transplantation may be used with subsequent division of the pedicle. This technique has been used by Dunphy and Keeley (1940) in an interesting, but somewhat incomplete, study of the function of the denervated adrenal gland in dogs.

Effect of Changes in Environmental Temperature

Pedicle transplantation without subsequent division of the pedicle provides a means of studying the effect of environmental change without denervating the viscus or interfering even temporarily with its blood supply. The best known example is Moore's (1922, 1924b) use of pedicle transplantation of the testis in his studies on the effect of temperature on testicular function (p. 504).

Transplantation in Endocrinology

Transplantation, and in particular free transplantation, has important applications in various fields of endocrinological research. We shall consider three examples: the control of anterior pituitary secretion, the inactivation of hormones by the liver, and the effect of hormones on tissues.

Control of Anterior Pituitary Secretion

The existence of a system of vessels linking the pituitary to the hypothalamus was first reported by Popa and Fielding (1930, 1933), who suggested that these vessels carried blood from the pituitary to the hypothalamus. Later investigations (*see* Harris, 1951, 1955 for review) suggested that the blood flow was actually in the opposite direction, and led to the hypothesis (Harris, 1944; Green and Harris, 1947, 1949) that the hypothalamus controls the function of the adenohypophysis (anterior pituitary) by a neuro-vascular link, hypothalamic nerve fibres on excitation liberating into the portal vessels a chemotransmitter capable of exciting or inhibiting the *pars distalis* of the gland.

Strong evidence in support of this hypothesis was obtained by Harris and Jacobsohn (1951, 1952), who found in rats that pituitary autotransplants placed under the median eminence of the tuber cinereum, where they acquired vascular connexions with the primary plexus of the hypophyseal portal vessels, showed normal functional activity, as judged by their effects on the adrenal glands and gonads, in at least 50 per cent of the animals, and somewhat diminished activity in the remainder. Transplants placed elsewhere, for example in the emptied hypophyseal capsule, under the temporal lobe of the brain, and in more distant sites, which become vascularized *but not by the hypophyseal portal vessels*, could be

maintained for long periods but were functionally ineffective

Experiments in rabbits (Jacobsohn, 1954) have confirmed the conclusion that the functional activity of anterior pituitary transplants is contingent on their acquiring connexions with the hypophyseal portal vessels

Inactivation of Hormones by the Liver

Golden and Sevringhaus (1938) transplanted the ovaries to the mesentery in rats and found that the transplants survived but the animals remained anoestrous The explanation of the phenomenon appears to be that the secreted oestrogen is inactivated in the liver, because spayed rats with pellets of crystalline oestrogens implanted in the spleen remain anoestrous likewise.

Biskind and Biskind (1944, 1945) carried the matter a stage further and showed that, except when adhesions formed which permitted anastomoses to develop between vessels of the transplant and extra-portal vessels of the host, autotransplants of ovary, and homotransplants of infantile testis, to the spleens of castrated rats underwent marked hypertrophy and sometimes formed tumours They suggested that this occurred because the transplants were subjected to protracted stimulation by the uninhibited pituitary of the host

These findings have been confirmed and extended by many investigators In rats and mice not only benign but also malignant tumours have been observed to develop in intrasplenic ovarian transplants (p. 496), and in rabbits testicular transplants having their venous drainage exclusively to the portal system have been shown to be endocrinologically inactive (p. 505). In monkeys, on the other hand, menstruation has been found to occur in castrated females bearing intrasplenic ovarian grafts (p. 496).

Experiments with thyroid transplants have yielded conflicting results (p. 484) but on balance appear to support the view that the thyroid secretion is partly inactivated by passage through the liver. Adrenal transplants draining to the portal system, however, appear to be functionally effective (p. 479).

Effect of Hormones on Tissues

The capacity of a hormone to stimulate a particular tissue can sometimes be demonstrated by establishing an autotransplant of the tissue in the anterior chamber of the eye and observing an increase in size and vascularity after administration of the hormone. This procedure might conceivably be made the basis of a method of bio-assay.

The effect of hormones circulating in the body may be studied in the same way. Autotransplants of endometrium in the anterior chamber, for example, undergo periodic changes corresponding to the oestrous cycle, becoming increasingly vascular and showing rhythmic capillary contractions during the oestrous phase in intact, but not in spayed, animals (Schochet, 1929; Markee, 1932).

CHAPTER 11

Transplantation as an Instrument of Research
II. Experimental Pathology

In this chapter we shall consider the application of transplantation in four fields of research

1 The study of tissue equilibrium and its regulation.

2 The study of specific immunological tolerance.

3 Cancer research

4 Investigation of the pathogenesis of certain disorders of the skin

TISSUE EQUILIBRIUM AND ITS REGULATION

Transplantation is useful in the study of tissue equilibrium in three ways

Firstly, in studying the processes of regeneration and repair, extirpation experiments may be supplemented by experiments with free autotransplants As we have seen (Chapter 2) free transplants, if larger than a certain critical size, undergo central necrosis, and when this occurs the ultimate fate of an autologous transplant depends largely on the extent to which regeneration occurs from viable tissue at the periphery This in turn depends partly on the nature of the tissues concerned, and partly on environmental factors, such as hormonal stimulation (*see*, e g , Ingle and Cragg, 1939)

Secondly, it is of interest to determine the extent to which disturbances of equilibrium due to loss of tissues can be corrected by transplantation

Experiments of this kind in amphibian larvae have shown that regeneration may be suppressed completely by promptly replacing an amputated limb by a limb homotransplant which fits the defect accurately and has the correct axial orientation (Weiss, 1923, 1925), or alternatively by covering the defect with an accurately fitting *homograft* of skin (Harrison, 1907, Detwiler, 1922), regeneration is not suppressed, however, if the transplant is a poor fit or if transplantation is not performed until regeneration is already under way

In adult mammals the capacity for regeneration is very limited but the process is still subject *to modification* by transplantation This is illustrated by the effect of skin autografts on the healing of raw surfaces A full thickness skin defect, if not too large, may heal spontaneously by a combination of fibroplasia, epithelial regeneration, and indrawing of the margins of the defect owing to contraction of fibrous tissue in its base The process of contraction, though an aid to healing, may cause serious deformity (Chapter 13) If, however, the defect is produced aseptically, and is covered immediately with an autologous skin graft, the amount of contraction is much less, and with a full thickness or nearly full thickness graft may be negligible Similarly, as Gillman Penn, Bronks and Roux (1953) have

shown, the healing of partial thickness defects is modified if they are covered with grafts.

Thirdly, tissue equilibrium may be deliberately disturbed by transplantation with the object of observing the interaction of the transplant and its environment. When, as sometimes happens, the structure of either the transplant, the surrounding tissue, or both is altered in some specific way in consequence of the changed tissue relationships the phenomenon may properly be termed *induction*.

The most spectacular instances of induction occur in embryonic and multipotent regenerating tissue but the process may also occur in apparently stable adult tissues. Induction in embryonic tissue has been considered at length in the preceding chapter, and two important examples of induction in adult mammalian tissues have been discussed in Chapter 2. Induction in regenerating tissue is most easily demonstrated in urodeles. If, for example, the tail of a salamander is amputated, and subsequently the mass of proliferating cells known as the *blastema* which forms at the amputation site is transplanted to a new site near the base of a forelimb,* then provided that the interval between amputation of the tail and transplantation of the blastema is less than a certain critical period a limb, not a tail, is formed (*see* Weiss, 1939, p. 476).

*Some limb nerve must be deviated towards the transplant. If this is not done regeneration will not occur (*see* Weiss, 1939, p. 446).

SPECIFIC IMMUNOLOGICAL TOLERANCE

The discovery of methods of inducing specific immunological tolerance to homografts (Chapter 7) has stimulated a great deal of important work with other antigens* including micro-organisms (Buxton, 1954; Kerr and Robertson, 1954; Cohn, 1957) and proteins (Hanan and Oyama, 1954; Cinader and Dubert, 1955, Dixon and Maurer, 1955; Cohn, 1957, Hort and Hraba, 1957; Cinader and Pearce, 1958, Stevens, Pietryk and Ciminera, 1958). It has also helped to orientate thinking on apparently related phenomena such as Felton's immunological paralysis (Felton and Ottinger, 1942; Felton, Prescott, Kauffmann and Ottinger, 1947; Felton, 1949; Felton, Kauffmann, Prescott and Ottinger, 1955), and the Sulzberger-Chase phenomenon (Sulzberger, 1939; Chase, 1946, 1949).

In the special case of experiments with the Rous chicken sarcoma virus, transplantation has not only suggested a profitable line of investigation but has formed part of the experimental procedure. In brief it has been shown by Simonsen (1955) that whereas normal turkeys cease to be susceptible to the Rous tumour during the second or third week after hatching, turkeys which have been injected as embryos with chicken blood may develop sarcomas when injected at the age of three weeks with a cell-free preparation of the virus, and he has suggested that this observation indicates an altered virus-host relationship resulting from the fact that the turkeys had been rendered tolerant to chicken tissue.

*Previously Burnet, Stone and Edney (1950) had tried unsuccessfully to induce specific tolerance by injecting chick embryos with *E. coli*, heterologous erythrocytes, bacteriophage and type A influenza virus.

CANCER RESEARCH

Transplantation has many applications in cancer research. We shall consider firstly transplantation of normal tissues and secondly transplantation of tumours.

Transplantation of Normal Tissues

In the first place, as we have already seen, transplantation is one means of studying the factors controlling tissue equilibrium. There are those who believe that one could know all about normal growth and the factors controlling it without having the least understanding of neoplastic growth (cf. Foulds, 1940, Oberling and Guérin, 1951), but whether this be true or not it would seem that understanding of neoplastic growth must be founded on knowledge of normal growth processes. As Nicholson (1938) pertinently asks. "Who . . ., *ignorant of a mechanism, reasonably hopes to show precisely where or how it has gone wrong?*"

Secondly, transplantation has proved useful in studying the action of carcinogenic agents, especially on embryonic tissues.

Smith and Rous (1945a), in the first experiment of this kind, transplanted mouse embryo skin together with oil containing Sharlach R and methylcholanthrene to adult mice of the same strain, and found that epithelial lined cysts containing the mixture were formed and soon gave rise to tumours. In later experiments (Smith and Rous, 1945b, 1946, Smith, 1947) they used a similar technique to produce tumours from embryo gastric epithelium, lung, ovary, and bile duct epithelium.

Greene (1953c, d) studied the behaviour of homotransplants and heterotransplants of embryonic rabbit and rat skin treated with material containing the Shope rabbit papilloma virus. Typical papillomata developed in embryonic rabbit skin transplanted homologously to the brain, the anterior chamber of the eye or the subcutaneous tissue of adult rabbits, or heterologously to the brain or anterior chamber of mice, and in embryonic rat skin transplanted homologously to the brain of adult rats. The last of these findings is of special interest because adult rat skin appears to be completely resistant to the Shope virus.

Billingham, Orr and Woodhouse (1951) studied the behaviour of epidermal, split-skin* and whole thickness grafts of methylcholanthrene treated adult skin transplanted orthotopically to untreated body sites, and also of untreated skin transplanted to denuded methylcholanthrene treated areas. They found that treated whole-thickness and thick split grafts yielded a small number of tumours when transplanted to untreated sites, whereas thin split grafts and epidermal grafts yielded no tumours. *On the other hand a considerable number of tumours resulted when untreated* epidermis was transplanted to a denuded carcinogen treated area. It would seem, therefore, as Billingham *et al.* cautiously suggest, that "the effective carcinogenic action of methylcholanthrene is not limited to the epithelium itself, but that the changes in the deeper tissues are of great importance."

Transplantation of Tumours

It was shown many years ago by Hanau (1889) and Jensen (1903) that cancer could sometimes be transmitted from one animal to another by transplantation, and the large number of transplantable tumours now maintained in laboratories all over the world bears witness to the importance of this discovery.†

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* *The term split skin graft is used here in accordance with the definition given on page 227*

†Dunham and Stewart (1953) have published a useful list of transplantable tumours available in various species.

Until recently all the tumours maintained by transplantation have originated in animals (as distinct from human beings) either spontaneously or in consequence of treatment with a carcinogen, and they have been propagated only in the species, and often only in the strain, in which they arose. As we have seen (p. 152), however, a number of human tumours have now been successfully transplanted heterologously to laboratory animals conditioned by irradiation or administration of cortisone, and propagated by serial transplantation in the new species.

Some transplantable animal tumours are propagated by solid transplants. Others, such as the Ehrlich mouse ascites tumour and other tumours with similar properties, are most conveniently propagated by cell suspensions (Loewenthal and Jahn, 1932, Warren and Gates, 1936; Seeger, 1937; Klein and Klein, 1951, Klein, 1953; Klein, 1954, Baillif, 1954). Large doses are normally used, but some ascites tumours will grow after inoculation of less than a hundred cells (Kahn and Furth, 1938, Reinhard, Goltz and Warner, 1945) and one at least has been transmitted by inoculation of two cells (de Ropp and McKenzie, 1954), while experimental leukaemia has been transmitted by means of a single cell (Furth and Kahn, 1937). Finally, there are some tumours such as the Rous chicken sarcoma and the Shope rabbit papilloma which can be transmitted by cell-free extracts (p. 202).*

Transplantation, it has been said, gives a tumour the advantage of immortality, and transplantable tumours have many applications in cancer research. They are of special importance in the study of tumour-host relationships, the role of viruses in the etiology of cancer, and tumour immunogenetics; in searching for and testing new methods of treatment; and as a source of material for morphological studies, and for biochemical and biophysical investigations *in vitro*. We shall consider some examples under each of these headings.*

*Gross (1951) has reported that a proportion of C3H mice injected within twelve hours of birth with either (a) Ak leukaemic cells, (b) Ak embryo tissue or (c) centrifuged extracts of Ak leukaemic tissue develop generalized leukaemia some months later, whereas older C3H mice are not susceptible to this leukaemia. He has therefore suggested that "spontaneous mouse leukaemia may be caused by an agent which is transmitted from one generation to another, like that of chicken lymphomatosis."

Tumour-Host Relationship

In this section we shall consider briefly the light thrown by transplantation on four aspects of the tumour-host relationship: (a) the extent to which tumour growth is independent of the restraints which limit the growth of normal tissues; (b) the phenomenon of progression; (c) the occurrence and distribution of metastases; and (d) systemic effects caused by tumours.

Autonomy and Dependence. It was postulated long ago by Ribbert (1895) that tumour formation can result from loss of growth restraint by adjacent tissue. Transplantation experiments soon showed, however, that normal cells transplanted to abnormal sites do not behave as cancer cells, and that when tumours are transplanted homologously to normal hosts the cancer cells may proliferate though the stroma degenerates and is replaced by connective tissue and vessels from the host (Bashford, Murray and Cramer, 1905; Ehrlich, 1907; Russell, 1908a); it was therefore concluded that, as Murray (1933) has expressed it, "the agencies which are effective in the body in limiting the rate and amount of growth and cell division are ineffective against true new growths."

Further work has shown that this conclusion is too sweeping and that, while Ribbert's concept in its original limited sense is probably untrue, the growth of some tumours is dependent on special environmental factors, of which continued hor-

*To keep the discussion within reasonable bounds no attempt has been made to cite all the original authorities when adequate reviews are available.

monal stimulation is the best understood Thus thyroid tumours induced by a goitrogen can at first be successfully transplanted only to hosts whose own thyroids have been similarly blocked (Bielschowsky, Griesbach, Hall, Kennedy and Purvis, 1949), though after several passages they may become capable of surviving in untreated recipients of the same inbred line (Morris and Green, 1951) Again, chromophobe pituitary adenomas induced in rats by oestrogens are transplantable to oestrogen treated rats but not to untreated rats of the same inbred line (Dunning, Curtis and Segaloff, 1947) These and many other examples are cited by Furth (1953) and Bielschowsky (1955) in their excellent reviews of the problem

Tumours whose growth is conditional on lack of restraint or on continued hormonal stimulation are often described as *dependent*, in contrast to *autonomous* tumours which are not subject to these limitations The distinction is a relative, not an absolute, one, provided this is understood, however, the terms are legitimate and useful

Another criterion of autonomy has been proposed by Greene (p 141), namely, ability to grow when transplanted heterologously to either the anterior chamber of the eye or the brain of a suitable host This criterion, though somewhat arbitrary, appears at first sight to have the merit of being perfectly clear cut In fact, as we have seen, the factors which determine the fate of tumour heterotransplants have not been fully elucidated and the results obtained by different investigators show wide variation Generally speaking, however, tumours which possess a high degree of autonomy in the sense in which we have used the term are more likely to prove *heterotransplantable* to special sites, or to appropriately conditioned hosts, than those which *do not*.

Progression. The term progression, originally used by Rous and Beard (1935) to denote the change of a virus induced rabbit papilloma into a carcinoma, has been defined by Foulds (1951, 1954) as "the development of a tumour by way of permanent, *irreversible qualitative* change in one or more of its characters" The change from a phase of dependence to one of autonomy, discussed in the preceding section, clearly falls within the scope of this definition

Transplantation is useful in the study of progression in two ways In the first place *progression*, as *Foulds* (1954) *points* out, "does not always reach an end point within the life span of the host," and transplantation "*gives extra time for the action of etio*logical agents, hastens by selection the dominance of the most resistant or most aggressive members of a mixture of varied cells, and extends the opportunities for new changes" (Foulds, 1954 *See also* Barrett and Deringer, 1950, Barrett, Deringer and Hansen, 1953) Secondly, transplantation provides a valuable test of reversibility (*see* Barrett and Deringer, 1952)

Metastasis. In studying the phenomenon of metastasis, transplantation has been used in three different ways

In the first place it has been used to detect the presence of metastatic tumour cells As long ago as 1889 Hanau detected metastases in the lymph nodes of a rat by subcutaneous transplantation to other rats The method was subsequently used by various investigators (for review *see* Goldie, Jeffries, Jones and Walker, 1953), but the results were variously interpreted, development of tumours after transplantation of apparently normal tissue being sometimes attributed to a cancer virus (Blumenthal and Auler, 1927) Eventually it was shown by Woglom (1940) that even a few metastatic tumour cells in apparently normal tissues can account for the growth of tumours after transplantation Goldie *et al.* (1953) have confirmed this and have shown that intra-

peritoneal inoculation of minced tissue constitutes a particularly sensitive test.

Secondly, the factors which govern the distribution and number of metastases have been investigated by injecting tumour cells in suspension either (a) intravenously, (b) into the arterial side of the circulation, or (c) into lymphatic vessels.

Intravenous injection normally gives rise to tumours mainly, if not exclusively, in the lungs. If, however, cortisone is administered concomitantly with the intravenous injection of tumour cells there may be a widespread development of tumours throughout the body (Pomeroy, 1954) It would seem that a small proportion of the injected tumour cells pass the filter, but most if not all of them are destroyed unless the host's resistance is depressed by cortisone.

Injection into the arterial side of the circulation has been studied by Coman and his colleagues (Coman, Eisenberg and McCutcheon, 1949, Coman, De Long and McCutcheon, 1951; Coman, 1953) Their findings suggest that the incidence of metastases of a given tumour in any organ is proportional to the number of tumour emboli lodging in the capillary circulation of that organ, they thus provide a serious challenge to the so-called soil theory (Willis, 1948), which has been widely accepted as a sufficient explanation of the distribution of metastases ever since it was propounded by Paget in 1889.

Injection into lymphatic vessels has been used by Zeidman and Buss (1954) to assess the efficiency of the corresponding lymph nodes as a barrier to the further passage of tumour cells.

Finally, observations have been made on the development of metastases from transplanted tumours located in various sites. This is exemplified by the work of Plenk, Sorenson and Eichwald (1954), who investigated the time interval between transplantation of a tumour to the anterior chamber of the eye and metastasis to lymph nodes by removing the eyes of tumour-bearing hosts at intervals and observing the animals for two months thereafter to see whether lymph node metastases become apparent.

Systemic Effects. Malignant tumours, whether spontaneous, induced or transplanted, cause profound alterations in the metabolism of the host; among these, changes in nitrogen metabolism (for review *see* Fenninger and Mider, 1954) and in liver catalase activity (*see* Greenstein, 1947) have aroused special interest. In recent years a transplantable rat tumour known as the Walker carcinoma 256 has been widely used in metabolic studies, and experiments with this tumour will serve as an illustration of the use of transplantable tumours in this field. The changes which have been demonstrated in animals bearing this tumour include anorexia (Mider, Tesluk and Morton, 1948); increased energy expenditure (Mider, Fenninger, Haven and Morton, 1951); loss of body weight (Mider, Tesluk and Morton, 1948) despite forced feeding (Stewart and Begg, 1953a), associated with loss of nitrogen (Mider, Tesluk and Morton, 1948, Stewart and Begg, 1953b) and lipoid (Mider, Sherman and Morton, 1949) from the body; and decreased liver catalase activity, anaemia, and enlargement of the adrenals (Begg and Dickinson, 1951).

Special systemic effects may be produced by tumours of endocrine organs (for review *see* Foulds, 1940; Woolley, 1950; Wollman, Morris and Green, 1951).

The Role of Viruses in the Etiology of Cancer

It was suggested by Borrel in 1903 that cancer was due to virus infection, but this hypothesis was entirely without [illegible] tal foundation until some years later when Rous (1910b, 1911) described a [illegible] fibromyxosarcoma which could be transmitted not only by transplantation but also

by injection of a cell free filtrate of tumour tissue.

Since then the Rous sarcoma has been made available to, and propagated in, laboratories all over the world, and the agent responsible for its transmission has been studied in great detail, in addition other tumours transmissible by cell free filtrates have been discovered not only in birds but also in amphibia and mammals (for review *see* Foulds, 1934a, b, Oberling and Guérin, 1954) The majority of tumours, especially of mammalian tumours, have not proved transmissible except by cells, and the hypothesis that *tumours in general are due to* infection with an exogenous virus is now rejected by all but a few devoted adherents If, however, the term virus is *understood* to include not only exogenous submicroscopic particles capable of entering and reproducing in cells but also self reproducing particles originating in host cells such as, for example, the plasmagenes postulated by Darlington (1948), judgment on the hypothesis must be suspended until further experimental evidence is obtained

Tumour Immunogenetics

The genetics of homologous transplantation has been discussed in Chapter 1, and the immunological consequences of transplantation when the donor possesses histocompatibility genes which are not shared by the host have been examined in Chapter 5 As we have seen, transplantation of tumours has played an important part in this work and has contributed much to our knowledge of the mechanisms involved

The contribution of immunogenetic studies to an understanding of the nature and properties of tumours is less easy to define As we have seen, despite some claims to the contrary, there is overwhelming evidence that the development of immunity to a tumour transplant is contingent on, and to a large extent the consequence of, genetic differences between donor and host.

The qualification *to a large extent* is necessary for two reasons

In the first place the genotype of the predominant tumour cells may differ from that *of the host in consequence of mutations*, and the position may be further complicated by chromosome polyploidy (Hauschka and Levan, 1953)

Secondly, it is not yet certain whether there is, or is not, a component of the immunity which is not genetically determined but is evoked by and directed against the tumour by virtue of the fact that it is a tumour and not a transplant of normal tissue In other words it is uncertain whether or not there are antigens characteristic of cancer *per se*

This question is clearly of great importance It can best be attacked not by an exclusive study of tumour immunogenetics but by extending these studies to include observations on the behaviour of homotransplants of normal tissues under a wide variety of genetically defined conditions It might also be profitable to repeat and extend the immunological studies undertaken by Lumsden and others with tumour heterotransplants (Chapter 8)

Experimental Cancer Therapy

In recent years much work has been done with the object of finding new methods of treating cancer, to supplement, and perhaps replace, *surgical excision* and *established* forms of radiotherapy

The only way of proving that any given *procedure is effective against human cancer* is by clinical trial, but experiments with transplantable tumours and other tests are used, *with varying degrees of success, in the* hope of eliminating many of the methods which would in fact prove ineffective while conserving all of real value, and thus reducing the number of methods to be tried clinically to a practicable figure

To illustrate the value and also the limitations of tests using transplantable tumours

we shall consider a variety of proposed methods of treatment arranged under the following headings: chemotherapy, including administration of hormones and antibiotics; gonadectomy, adrenalectomy and hypophysectomy; infection with viruses and other micro-organisms, ligation of blood vessels, and exposure of the tumour-bearing animal to anoxia. Another procedure, namely total body irradiation followed by transplantation of haemopoietic tissue, is discussed in Chapter 22.

Chemotherapy. Transplantable tumours* provide a convenient, and within limits a useful means of testing substances for anti-tumour activity. Homotransplants in inbred strains of mice and rats are most widely used but *heterotransplants of human tumours established in laboratory animals* by the methods described earlier (p. 152) seem likely to play an important role in future. Heterotransplants on the chorio-allantoic membrane or in the yolk sac of the chick embryo have also been used, but have the disadvantage that they permit only a very short *period of observation.*

Various criteria have been used to assess [illegible] efficacy of the treatment, including [illegible]scopic and microscopic evidence of regression of the tumour (Shear, Hartwell, Peters, Dalton, Dunn, Hauschka, Rees, Dil-[illegible], Beck, McConnell, Holloman, Oakey and R[illegible]mann, 1947), tumour weight (Haddow and Robinson, 1937; Walpole, 1951) or size (Stock and Rhoads, 1949), viability of tumour cells as judged by retransplantation (Burchenal, Cremer, Williams and Armstrong, 1951), and the period of survival of *the host* (Flory, Furth, Saxton and Reiner, 1943; Law, 1947; Burchenal, Lester, Riley and Rhoads, 1948). Ascites tumours are especially useful when cytological observations or tests of cell viability are to be made because cell samples may readily be obtained by aspiration (Klein, 1950).

Many thousands of substances have now been tested. They comprise *synthetic compounds,* including nitrogen mustards and related substances, biological antagonists of folic acid and other vitamins, synthetic purine analogues, steroids, and urethane and other carbamates; *radioactive isotopes; materials of animal origin,* including spleen extracts, sera, enzymes, hormones, and unidentified substances obtained from urine; *substances obtained from plants,* including colchicine and podophyllin; and *substances obtained from micro-organisms and fungi,* including bacterial polysaccharides and many antibiotics. Useful general reviews of the findings have been published by Gellhorn and Jones (1949), Karnofsky (1952), Gellhorn (1953), and Stock (1954), and a bibliography appeared as a supplement to *Cancer Research* (see *Cancer Research*, 1956). The effect of growth hormone and *adrenocortical hormones, and of substances* obtained from micro-organisms, have been reviewed by Reid (1954) and Reilly (1953) respectively.

It is an indication of the difficulty of the problem, and the inadequacy of present screening tests, that from all this work only a few substances have been discovered which are of real clinical value, and not one of them is curative. Moreover, among these substances only two, urethane (Haddow and Sexton, 1946) and TEM (2,4,6-triethyleni-mino-S-triazine) (Buckley, Stock, Crossley *and Rhoads,* 1950; Burchenal, Crossley, Stock and Rhoads, 1950; Lewis and Crossley, 1950), were first shown to be active in experiments with animal tumours (Gellhorn, 1953). Oestrogens, which have proved so valuable in the treatment of carcinoma of the prostate (Herbst, 1941; Huggins and Hodges, 1941) and disseminated post-menopausal *carcinoma of the breast* (Haddow, Watkinson and Paterson, 1944; Gellhorn and Jones, 1949; Council on Pharmacy

*Including transplantable leukaemias (Flory, Furth, Saxton and Reiner, 1943, Law, 1947a, Burchenal, Lester, Riley and Rhoads, 1948).

Chemistry 1951 Gellhorn 1953) and androgens which are widely used in young and middle aged patients with disseminated carcinoma of the breast (Adair and Herrmann 1946 Adair 1947 Gellhorn and Jones 1949 Council on Pharmacy and Chemistry 1951 Lewison and Chambers 1952 Gellhorn 1953) had not been shown to have any effect on animal tumours until very recently when Huggins Briziarelli and Sutton (1959) succeeded in inducing mammary carcinomas in rats by administration of methyl cholanthrene through a stomach tube and showed that many of them were hormone dependent and the action of nitrogen mustards synthetic purine analogues and cortisone * which have been widely used in the palliative treatment of human reticuloses (for review see Gellhorn and Jones 1949 Karnofsky 1952 Gellhorn 1953) was demonstrated first in man and only later confirmed in experimental animals

Gellhorn (1953) has pointed out that compounds shown to possess anti tumour activity by tests with transplantable tumours are effective only against the most rapidly growing human tumours and he believes that the tests may be missing compounds which are incapable of producing a dramatic effect in a short time but would nevertheless significantly modify tumour growth if administered for long periods He suggests that to overcome this difficulty it may be necessary to adopt more subtle criteria of anti tumour activity than those in use at present

Klass (1956b) on the other hand has suggested that transplantable animal tumours are unsuitable for assessing the chemotherapeutic value of substances intended for use in human cancer because the graft host relationships of transplantable tumours differ so greatly from those of autochthonous tumours

Gonadectomy, Adrenalectomy and Hypophysectomy It is now established that life may be prolonged and symptoms alleviated in many patients with carcinoma of the prostate or disseminated carcinoma of the breast by gonadectomy and that a proportion of the patients who fail to respond to this treatment may be helped by adrenalectomy or in the case of patients with cancer of the breast by hypophysectomy

It is instructive to consider how these methods have evolved

Adenocarcinoma of the prostate has not yet been induced in animals nor has it been found to occur spontaneously except in dogs and monkeys Orchidectomy together with oestrogen administration was tried in patients by Huggins and his colleagues (Huggins and Hodges 1941 Huggins 1942 1946) firstly because it was known that the functional activity of normal prostatic epithelium was increased by administration of androgens and decreased by castration and secondly because they were able to show that in one respect at least namely production of acid phosphatase malignant prostatic epithelial cells displayed functional activity of a kind characteristic of normal cells

Oophorectomy was first performed in patients with inoperable carcinoma of the breast by Beatson (1896) and orchidectomy for carcinoma of the male breast by Farrow and Adair (1942) Beatson s work was soon forgotten more recently however the discovery that the incidence of mammary carcinoma in certain strains of mice is reduced by oophorectomy in early life (Loeb 1919) and conversely that administration of oestrogens to mice under certain conditions evokes the formation of mammary carcin

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* [illegible] which once appeared promising [illegible] On the other hand [illegible] and related [illegible] have been shown to cause temporary regression [illegible] and Kendall 1951 [illegible] Walker 256 rat carcinoma [illegible] and Rice 1950 [illegible]

oma (Lacassagne, 1932; Cramer and Horning, 1936; Shimkin, 1946; Mühlbock, 1956 [review]), has stimulated further clinical investigation, and has been largely responsible for the re-introduction of oophorectomy and for the development of androgen therapy in human breast cancer.

Bilateral adrenalectomy was performed for the first time in four patients with carcinoma of the prostate by Huggins and Scott (1945) in the belief that failure to respond to orchidectomy and administration of stilboestrol was due to production of androgens by the adrenals. One patient survived for 116 days, and growth of the tumour appeared to be retarded, but the other three died soon after operation owing to the inadequacy of the replacement therapy available at the time. Cox (1947) attempted to circumvent this difficulty by performing subtotal adrenalectomy but the effect on the tumour was only temporary. The discovery of cortisone changed the situation and encouraged further trial of adrenalectomy in patients with prostatic cancer, advanced mammary cancer, and a variety of other tumours including squamous-cell carcinoma, malignant melanoma and chorion-epithelioma. Regression has been found to occur in some prostatic and mammary carcinomas but in no other human tumours (Huggins and Bergenstal, 1951, 1952; Huggins and Dao, 1952; West, Hollander, Whitmore, Randall and Pearson, 1952; Huggins, Bergenstal and Cleveland, 1953; Taylor, Li, Eckles, Slaughter and McDonald, 1953; Cade, 1954; Pyrah and Smiddy, 1954).

Hypophysectomy was introduced by Luft and Olivecrona (1953) as an alternative to adrenalectomy for the treatment of mammary cancer. The rationale of the operation is that it causes atrophy of the gonads and adrenals, and in addition eliminates pituitary hormones acting directly on the mammary gland.

Experiments with transplantable animal tumours, while they have not played any significant part in the evolution of the methods of treatment we have been considering, have yielded results of considerable interest. Adrenalectomy increases the susceptibility of rats to transplantable leukaemia (Murphy and Sturm, 1943; Sturm and Murphy, 1944), and increases the incidence of spontaneous leukaemia in mice (Law, 1947b); but on the other hand it retards the growth rate of several other tumours in these species including transplantable sarcomas (Joannovics, 1916; Roffo, 1930) and the Walker 256 rat carcinoma (Funk, Tomashefsky, Soukup and Ehrlich, 1951; Ingle and Baker, 1951). It has been shown further that growth of the Walker 256 carcinoma is also retarded by hypophysectomy (Ball and Samuels, 1938; Funk, Tomashefsky, Ehrlich and Soukup, 1950; Funk, Tomashefsky, Soukup and Ehrlich, 1951; Talalay, Takano and Huggins, 1952; Huggins, Bergenstal and Cleveland, 1953). It is conceivable therefore that, as Huggins and Bergenstal (1952) have suggested, the beneficial effect of adrenalectomy and, it may be added, of hypophysectomy, in human prostatic and mammary cancer, while "due at least in part to elimination of critical amounts of sex hormones," may be partly "non-specific" in character.

The recent discovery by Huggins and his colleagues (p. 205) of a method of inducing hormone dependent mammary cancer in rats should open up new and exciting lines of investigation in this field.

Infection with Micro-organisms. Many attempts have been made to induce regression of tumours by infecting them with micro-organisms. Most of the experiments have been performed with transplantable tumours, and the agents used include bacteria, protozoa and filtrable viruses (for review see Reilly, 1953). We shall consider briefly some illustrative examples.

Beebe and Tracy (1907) claims to have obtained complete disappearance of tran

planted lymphosarcoma in dogs by injecting suspensions of *Bacillus prodigiosus*, either alone or together with streptococci Daels (1910) and more recently Comsia (1928) reported that infection with spirochaetes interfered with the development of tumour transplants in mice, and Parker, Plummer, Siebenmann and Chapman (1947) obtained temporary remission of the growth of a transplanted mouse fibrosarcoma by injecting spores of *Clostridium histolyticum* directly into the tumour and administering histolyticum antitoxin to protect the host

Regression of a number of transplantable mouse tumours has been obtained by infection with trypanosomes (Daels 1910 Roskin 1946 Klyueva 1947 Hauschka and Goodwin 1948) and some remission of leukaemia in mice by infection with a malarial parasite *Plasmodium bergai* (Nadel and Greenberg, 1953)

Affinity of a virus for tumour tissue was first demonstrated by Levaditi and Nicolau (1922) who showed that neurotropic vaccinia virus would multiply in various transplantable mouse and rat tumours Since then many other virus tumour combinations have been studied and it has been found that in a proportion of cases in which the virus survives the tumour grows more slowly or is actually destroyed (Levaditi Schoen and Reinie 1937, 1947 Schoen 1938 Andrewes 1940 Syverton and Berry 1947 Turner and Mulliken 1947a b 1950 Moore 1949a b 1951a b 1952, 1953 Koprowski and Norton 1950 Moore and O Connor, 1950 Sharpless Davies and Cox 1950 Kuwata 1951 Ginder and Friedewald 1951 1952 Koprowska and Koprowski 1953 Wagner 1954) The references just cited refer to experiments with animal tumours Toolan and Moore (1952) have reported however that Egypt virus 101 has a destructive effect on heterotransplants of a human epidermoid carcinoma maintained in irradiated rats

Unfortunately with a few exceptions (Sharpless *et al*, 1950 Ginder and Friedewald 1951, 1952 Moore, 1953), destruction of experimental tumours has been achieved only at the cost of eventual death of the host due to the virus infection Attempts to overcome this difficulty by immunizing the host in advance have proved futile because the tumours have then also been unaffected by the virus (Moore and O Connor, 1950) *It is this danger to the host which constitutes the main obstacle to the use of viruses in the treatment of human cancer* Despite the difficulties and dangers a few cautious clinical trials have been made (Bierman Hammon, Eddie Meyer and Shimkin, 1950 Southam and Moore, 1951, 1952 Higgins and Pack, 1951a, b) but so far no striking results have been obtained It would seem, nevertheless that this approach to the problem of the treatment of cancer is well worth pursuing

Ligation of Blood Vessels. Lewis and her colleagues (Lewis, Maxwell and Aptekman, 1951 Lewis and Aptekman, 1951, 1952) found that rats of an inbred strain which were susceptible to a transplantable carcinoma or sarcoma became resistant if an established transplant of the tumour in question was caused to atrophy by ligating the blood vessels supplying it Resistance was also induced in other hosts by transplanting to them necrosing tumour tissue produced in the same way On the other hand resistance was not conferred either by excising an established transplant or, as a rule, by transplanting viable tumour tissue to pockets in which the blood supply was restricted *ab initio*

The observation that resistance results from strangulation of an established tumour if confirmed in other species and with a variety of tumours including some which had originated spontaneously or been induced in the animal used in the experiment, would clearly be of great importance Far

don and Prince (1953) were unable to confirm it in mice, but more recently Strauss, Saphir and Appel, 1956) have reported long survival of human patients following electrocoagulation of rectal carcinomata and have attributed this to the action of substances absorbed from the necrotic tumour tissue. Until the matter has been further investigated it would seem unwise to draw any general conclusions.

Exposure to Anoxia. The effect of anoxia has been studied by exposing animals bearing tumour transplants to an atmosphere containing a reduced amount of oxygen (Campbell and Cramer, 1928, Pollack, Taylor and Sortomme, 1942, Barach and Bickerman, 1954). The growth rate of the tumours may be significantly retarded but unless special precautions are taken the hosts lose a good deal of weight. By using hosts which had been gradually acclimatized, or had been rendered thyroid deficient by administration of radioactive iodine, however, Barach and Bickerman (1954) were able to experiment with concentrations of oxygen well below the normal limit of tolerance; they thus obtained a marked retardation of tumour growth with either a slight fall in host body weight or, in some cases, no fall at all. These investigators made the further interesting observation that tumours maintained for a time in animals (mice) exposed to an atmosphere poor in oxygen and then transplanted to non-acclimatized hosts were no more tolerant of oxygen lack than tumours transplanted directly without such preliminary treatment. As far as these experiments go, therefore, it appears that, while animals become acclimatized to low oxygen tension, tumours fail to do so.

Transplantable Tumours as a Source of Material for Observations in Vitro

Transplantable tumours constitute an important source of material for morphological studies, investigations of the physical and chemical properties of tumour cells, observations on the metabolism and respiration of tumour cells *in vitro*, and tissue culture. Many examples are cited by Cowdry (1940) and Greenstein (1947); recent biochemical studies are well exemplified by the work of Weinhouse and his colleagues (Weinhouse, Millington and Wenner, 1951; Wenner, Spirites and Weinhouse, 1952; Wenner and Weinhouse, 1953; Medes, Thomas and Weinhouse, 1953; Weinhouse, Allen and Millington, 1953).

THE PATHOGENESIS OF CERTAIN DISORDERS OF THE SKIN

Transplantation has thrown considerable light on the pathogenesis of allergic eczema and certain other disorders of the skin, thanks largely to the work of Haxthausen (1943, 1944, 1947, 1948, 1951, 1953).

Allergic Eczema

There has been much discussion as to whether the hypersensitivity in allergic eczema is due to humoral antibodies formed elsewhere and conveyed to the skin by the blood stream, or to local processes in the skin in the form of either local antibody formation or a specific change in the reactivity of the cells to the allergen.

Bloch (1911) tried to answer this question by transplanting skin from a patient with iodoform eczema and also normal skin to a normal subject and performing patch tests seven days later, but his findings are of doubtful significance because both transplants were cast off soon after the patch tests were made. To overcome this difficulty Haxthausen (1943) made experiments with human identical twins. Two pairs of twins were used, one pair being denoted by

the letters X and Y, the other by A and B. X and A were deliberately sensitized by a single application of 2-4-dinitrochlorbenzene,* and some days after universal hypersensitiveness to this substance became manifest skin was grafted† from X to Y and *vice versa*, and from A to B and *vice versa*. Three weeks later tests were made by painting the grafts and also host skin in the vicinity with a one per cent alcoholic solution of dinitrochlorbenzene. The two sensitized subjects X and A showed marked eczematous reactions *on the grafts* as well as on the surrounding skin, whereas no reaction occurred in Y and B even on the primarily-sensitized grafts. It was therefore concluded "that the hypersensitiveness *cannot be due* to a change in the reactivity of the epidermal cells or to a local antibody formation in the skin, and that it must be due to a factor conveyed to the skin *from* the sensitized organism, most likely a humoral antibody."

There seems no reason to suggest that the circulating cells of the sensitized individual play no part in the process, but with this proviso the conclusion seems reasonable. Supporting evidence was provided later by parabiosis experiments with guinea pigs in which it was found that hypersensitivity to dinitrochlorbenzene was transmitted from a sensitized animal to an untreated parabiont (Haxthausen, 1944).

For investigations in man the use of identical twins imposes a serious restriction, and more recently Haxthausen (1948, 1951) has experimented with very small homografts exchanged between unrelated individuals, and has succeeded in some instances in obtaining survival of sufficiently long duration to

tivity*.

in these experiments depends not so much, as Haxthausen appears to believe, on the small size of each graft, but on the smallness of the total "dose" (p. 15) of foreign skin.

Circumscribed Scleroderma, Vitiligo and Acrodermatitis Atrophicans

In patients with circumscribed scleroderma, vitiligo or acrodermatitis atrophicans, as Haxthausen (1947) has shown, split skin autografts taken from unaffected to affected areas soon show pathological changes characteristic of the disease, whereas grafts from affected to unaffected areas *gradually resume the structure of normal* skin. It would seem therefore that these disorders are the result of local trophic influences, possibly exerted through the autonomic nervous system.

Lentigo

In circumscribed congenital pigmentation (lentigo) Haxthausen (1947) found that *autografts* exchanged between *normal* and pathological areas retained their original characteristics.

Psoriasis

In preliminary experiments Haxthausen (1947, p. 367) found that when affected skin was grafted autologously to an unaffected area the graft usually preserved its pathological character, and sometimes psoriasis extended to the surrounding skin. Similarly, normal skin grafted to the centre of a psoriasis plaque usually became "invaded" by psoriasis, first at its periphery and later throughout its whole extent.

It will be seen that this process bears a striking resemblance to the phenomenon of pigment spread in guinea pigs described earlier (p. 38).

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*Haxthausen points out that dinitrochlorbenzene is not normally encountered except in certain special chemical industries, and that the sensitization evoked by a single application usually persists for only a few months: the risk of permanent harm to the subjects of the experiment was therefore very small.

†Haxthausen used *free split skin grafts* but he unfortunately refers to them as *flaps*. The usual meaning of these terms is explained in Chapters 13 and 14.

*Haxthausen (1951) suggests that some of his small grafts survive permanently, but he does not present convincing evidence of this.

CHAPTER 12

The Transplantation of Tissues and Organs in Surgery

General Considerations

THE SURGERY OF REPLACEMENT

Whenever important structures are congenitally absent, or are irreparably damaged by injury or disease, the problem of replacement arises. In many instances no solution can be found, for the surgery of replacement, though of ancient lineage, has developed very slowly in comparison with the surgery of ablation, but important advances have been made in recent years (Woodruff, 1952b, 1957e).

The methods of replacement which are available are as follows:

1. Transplantation of living tissue.
2. Transplantation of dead human or animal tissue.
3. Implantation of dead materials other than animal tissue, such as metals and plastics
4. Replacement by removable prostheses, for example dentures and artificial limbs.

This book is concerned primarily with transplantation but some reference will be made to the use of prostheses as substitutes for blood vessel (Chapter 21) and other transplants.

SURVIVAL AND UTILITY

In previous chapters we have been concerned mainly with transplants of living tissue and the conditions which determine their survival; in this and later chapters we shall be concerned with transplants of both living and dead tissue, and with their clinical usefulness.

There are three ways in which transplants may be of benefit to patients.

In the first place some transplants of living tissue, for example skin autografts, may survive throughout the life of the recipient, and thus provide permanent replacement of lost tissue.

Secondly, other transplants of living tissue, while not surviving permanently, may suffice to tide a patient over a period of crisis. Skin homografts, for example, though they survive for only two or three weeks, may provide valuable temporary cover in severely burned patients at a time when extensive autografting would be hazardous; and homotransplantation of a whole kidney by vascular anastomosis has been used successfully in cases of acute temporary renal failure as an alternative to dialysis with an artificial kidney.

Thirdly, other transplants again, including some transplants of living tissue, and probably all *useful* transplants of dead tis-

sue though eventually absorbed provide an effective scaffolding for the growth of host tissue Massive bone autografts homografts of bone and of blood vessels and possibly also homografts of cornea fall in this category

Transplants are sometimes characterized as *vital* or *static* according as their usefulness does or does not depend on their continued survival in particular homotransplants are subdivided into *homovital* transplants and *homostatic* transplants These terms which were introduced by Longmire (1952) are open to objection on etymological grounds, but are so convenient that they seem likely to gain general acceptance

THE PRESENT STATUS OF TRANSPLANTATION IN SURGERY*

Autotransplants

Free autotransplants of skin and of connective tissues including fascia dermis, fat, tendon bone and cartilage are commonly used and give excellent results under appropriate conditions Autologous vein transplants still have a place in vascular surgery for the reconstruction of peripheral arteries especially when arterial homografts and plastic prostheses are not available Nerve transplants are generally speaking less satisfactory but are of value in some instances

Free autotransplantation of endocrine tissue is indicated occasionally It is good practice for example if a parathyroid gland is removed accidentally during thyroidectomy and recognized at the time to cut it into small pieces and transplant these to the sternomastoid muscle It is also useful on occasion to transplant ectopic endocrine tissue for example lingual thyroid to a different site

Pedicle transplants of skin and subcutaneous tissue are widely used in reconstructive surgery The term pedicle transplantation is applied to viscera strictly speaking includes transplantation of a viscus from an abnormal site back to its normal site as in reduction of a hernia but we shall consider only transplantation of the whole or part of a viscus to an abnormal site A common example is transplantation of part of the stomach or a portion of jejunum to the chest to restore continuity after excision of the lower part of the oesophagus

Homotransplants

Homotransplantation of cornea is a well established clinical procedure and at present provides the only means of restoring sight in patients blinded by irreversible opacity of the cornea Homotransplants of stored bone and cartilage are used extensively in some clinics in place of autotransplants

Arterial homotransplants have been, and are still being used in the treatment of lesions of the aorta and the larger peripheral arteries although in many clinics they are being displaced by prostheses A few years ago it looked as if the use of artery grafts and prostheses in obliterative disease of the peripheral arteries would soon be abandoned but the development of shunt grafts (p 123) has given a new impetus to this branch of surgery

Homotransplants of skin are being used increasingly to provide temporary cover in severely burned patients Of all tissues, skin is probably the easiest to obtain and it can now be stored in a viable state for long periods

Homotransplants of whole kidneys by vascular anastomosis are, as we have seen, being used in a few clinics for the treatment of

*Only a brief review of the position is given here For further details the reader is referred to the chapters which follow

acute temporary renal failure, but it is difficult as yet to assess the value of the procedure. On the other hand isotransplantation of a kidney from an identical twin—if one is available and is willing to donate a kidney—is certainly justified in selected cases and some excellent results have been reported (Chapter 25).

Permanent functional recovery in patients with endocrine deficiencies following free homotransplantation of the appropriate tissue is still reported from time to time, but most observers remain sceptical about these claims.

Heterotransplants

Despite some moderately optimistic reports heterografts of blood vessels and bone are decidedly inferior to homografts and should be regarded as obsolete. The same is probably true of heterografts of cartilage though they are still used by some surgeons.

It appears just possible that heterotransplants of cornea will one day find a place in surgery, but it seems extremely unlikely that vital heterotransplants will ever be of therapeutic value.

SOURCES OF HOMOTRANSPLANTS OF HUMAN TISSUE

Autotransplants, though ideal in other respects, are often either not available in sufficient quantity, or obtainable only at considerable inconvenience to the patient. Removal of autologous bone and cartilage may add much to the burden he has to bear, and even in the case of skin, where autotransplantation finds its greatest application, so much may have been lost that there are not sufficient donor areas available to meet his needs. Heterotransplants, on the other hand, though easily obtainable, are often useless, and even when this is not the case they are less satisfactory than homotransplants. The task of obtaining human tissues for use as homotransplants is therefore of great importance, and if the biological problem which forms the central theme of the first part of this book is ever solved it will become more important still.

There are three possible sources of supply: voluntary donors, patients undergoing surgical operations, and cadavers. In each case the donor must be free from transmissible infectious disease including pyogenic infection, syphilis, tuberculosis, malaria, and infective hepatitis. In addition, although the risk of transmitting cancer accidentally by transplantation of apparently normal tissues is probably small, it is usual—and desirable—to insist that the donor is free of malignant neoplastic disease.*

Voluntary Donors

By using voluntary donors healthy normal tissue can be obtained with minimal risk of bacterial contamination. This procedure is justifiable however only under exceptional circumstances.

It would probably be generally agreed that it is reasonable to ask for volunteers from among a patient's relatives or friends to supply limited quantities of split-skin grafts when these may be life-saving and no other source of supply is available at the time. The author had occasion to do this once in treating a severely burned child, and found no lack of generous people willing to act as donors. It also seems reasonable to ask an identical twin if he would agree

*In using autografts there is a risk of transferring malignant disease to or from a donor site unless proper [illegible] he subsequently happen to develop cancer

to donate a kidney provided that his other kidney is normal and the patient is unlikely to recover without a transplant but is fit to undergo the operation

Tissue Removed at Operation

In some operations normal tissue is removed and this tissue being fresh and to all intents and purposes free from bacterial contamination is often valuable for transplantation Thus ribs removed during thoracoplasty constitute an important source of supply for the bone bank and healthy corneae can sometimes be obtained from eyes removed for various reasons In some hospitals where large numbers of infants are circumcised the foreskins have been collected and used as homografts other workers have used skin from amputated limbs for the same purpose Finally, foetal tissues if required can be obtained when pregnancy is terminated by hysterotomy

More commonly tissue removed at operation is pathological and it is then as a rule not useful for transplantation A possible exception was reported by Broster and Gardiner Hill (1946) who transplanted a hyperplastic adrenal gland from a patient with *virilism* to one suffering from Addison s disease It has not been proved by biopsy that this transplant survived and such operations are probably not justifiable in our present state of knowledge but if the homograft problem is solved hyperplastic endocrine tissues may become much in demand

Tissue Obtained Post Mortem

Legal Considerations

It has long been customary for tissues and organs of pathological interest to be removed at autopsy and preserved in museums The practice of removing normal tissues and organs including eyes to provide material for transplantation is of later origin however and cannot be said to have the sanction of long usage nor, until recently had it any legal sanction

This dangerous state of affairs has now been remedied in several countries

In France legislation was introduced in 1947 which permitted the removal of eyes under certain conditions to provide corneal grafts but it was not until 1952 with the passing of the Corneal Grafting Act that a similar step was taken in Great Britain In view of its great importance this Act is quoted in full

An Act to make provision with respect to the use of eyes of deceased persons for therapeutic purposes
(26th June 1952)

Be it enacted by the Queen s most Excellent Majesty by and with the advice and consent of the Lords Spiritual and Temporal and Commons in this present Parliament assembled and by the authority of the same as follows —

(Removal of eyes of deceased persons)

1 (1) If any person either in writing at any time or orally in the presence of two or more witnesses during his last illness has expressed a request that his eyes be used for therapeutic purposes after his death the party lawfully in possession of his body after his death may unless he has reason to believe that the request was subsequently withdrawn authorise the removal of the eyes from the body for use for those purposes

(2) Without prejudice to the foregoing subsection the party lawfully in possession of the body of a deceased person may authorise the removal of the eyes from the body for the purpose aforesaid unless that party has reason to believe—

(a) that the deceased had expressed an objection to his eyes being so dealt with after his death and had not withdrawn it or

(b) that the surviving spouse or any surviving relative of the deceased objects to the deceased s eyes being so dealt with

(3) An authority given under this section in respect of any deceased person shall be sufficient warrant for the removal of the

eyes from the body and their use for the purposes aforesaid, but no such removal shall be effected except by a registered medical practitioner, who must have satisfied himself by a personal examination of the body that life is extinct

"(4) Authority for the removal of eyes shall not be given under this section if the party empowered to give such authority has reason to believe that an inquest may be required to be held on the body

(5) No authority shall be given under this section in respect of the body of a deceased person by a person entrusted by another person with the body for the purpose only of its interment or cremation

(6) In the case of a body lying in a hospital, any authority under this section may be given on behalf of the person having the control and management of the hospital by any officer or person designated in that behalf by the first mentioned person.

(7) Nothing in this section shall be construed as rendering unlawful any dealing with or with any part of, the body of a deceased person which would have been lawful if this Act had not passed.

(8) In the application of this section to Scotland for subsection (4) there shall be substituted –

(4) Nothing in the foregoing provisions of this section shall authorise the removal of eyes from a body in any case where the procurator fiscal has objected to such removal."

(Short title, extent and commencement.)

"2 (1) This Act may be cited as the Corneal Grafting Act, 1952

"(2) This Act shall not extend to Northern Ireland.

"(3) This Act shall come into force three months after the passing of this Act."

The Corneal Grafting Act regularizes the position only in respect of the removal of eyes In practice in Britain it appears to be safe, though not legal, to remove other structures provided that permission for an autopsy has been obtained and the body is not disfigured apart from the usual incision.

In New Zealand an Act known as the Medical Amendment Act 1954 has been passed which authorizes the removal not only of eyes but of any tissues and organs under conditions similar to those laid down in the British Act. The New Zealand Act may well be taken as a model and for this reason its main provisions are quoted in full. They are as follows:

"(Removal of healthy tissue for therapeutic purposes. Cf. Corneal Grafting Act 1952 (15 & 16 Geo. VI & 1 Eliz. II, ch. 28) (U.K.))

"24A. (1) If any person, either in writing at any time or orally in the presence of two or more witnesses during his last illness, expresses a request that his eyes or any other part of his body be used for therapeutic purposes after his death, the person who is lawfully in possession of his body after his death may, unless that person has reason to believe that the request was subsequently withdrawn, authorize the removal of the eyes, or of any part of the body to which the request relates, for use for those purposes.

"(2) Without affecting the provisions of subsection one of this section, it is hereby declared that the person who is lawfully in possession of any body may authorize the removal of the eyes or of any other part of the body for use for therapeutic purposes, unless—

"(a) That person has reason to believe that the deceased person had expressed an objection to his eyes or any part of his body being so dealt with after his death; or

"(b) The surviving husband or wife or any known relative of the deceased person requires that the body be buried or cremated without such removal.

"(3) Subject to the provisions of this section, any authority given under this section in respect of any body shall be sufficient authority for the removal and use for therapeutic purposes of the eyes or of any part of the body to which it relates:

"Provided that no such removal shall be effected except by a registered medical practitioner who has satisfied himself, by a personal examination of the body, that life is extinct.

(4) If any person who is empowered under this section to give any such authority as aforesaid has reason to believe that an inquest may be required to be held on the body, he shall not give that authority without the consent of a Coroner, who may give his consent subject to such conditions (if any) as he thinks fit.

(5) No authority shall be given under this section in respect of any body by a person who is entrusted by another person with the body for the purpose only of its burial or cremation.

(Post mortem examination for research and medical education.)

24B. (1) If any person, either in writing at any time or orally in the presence of two or more witnesses during his last illness, expresses a request that after his death a post mortem examination of his body be performed for the purposes of medical research or the teaching of pathology, the person who is lawfully in possession of his body after his death may, unless that person has reason to believe that the request was subsequently withdrawn, authorize a post mortem examination of the body for any such purposes.

(2) Without affecting the provisions of subsection one of this section, it is hereby declared that the person who is lawfully in possession of any body may authorize a post mortem examination of the body for the purposes of medical research or the teaching of pathology if—

(a) That person has no reason to believe that the deceased person had expressed an objection to a post mortem examination; and

(b) The surviving husband or wife, or, if there is no husband or wife known to be surviving and there is known to be a relative, any such relative, consents to a post mortem examination.

(3) Subject to the provisions of this section, any authority given under this section in respect of any body shall be sufficient authority for the post mortem examination of the body and for the removal of any diseased tissue for the purposes of medical research or the teaching of pathology.

(See Reprint of Statutes, Vol. III, p. 725.)

(4) No post mortem examination shall be performed under this section except by the Medical Superintendent of an institution within the meaning of the Hospitals Act 1926, or by a registered medical practitioner who is a pathologist on the staff of any such institution (whether he is employed in an honorary capacity or otherwise), or who is a pathologist on the staff of any school of medicine or surgery at any constituent college of the University of New Zealand, or who is the holder of a licence for the time being in force under section twenty-four C of this Act. Before performing the examination the person who is to perform it shall satisfy himself, by a personal examination of the body, that life is extinct.

(5) If any person who is empowered under this section to give any such authority as aforesaid has reason to believe that an inquest may be required to be held on the body, he shall not give that authority without the consent of a Coroner, who may give his consent on and subject to such conditions (if any) as he thinks fit.

(6) No authority shall be given under this section in respect of any body by a person who is entrusted by another person with the body for the purpose only of its burial or cremation.

(Licences to perform post mortem examinations under last preceding section.)

24C. (1) The Minister of Health may from time to time grant to any registered medical practitioner a licence to perform post mortem examinations for the purposes of section twenty-four B of this Act.

(2) Any licence under this section shall continue in force until it is cancelled or surrendered:

Provided that any such licence may, if the Minister thinks fit, be granted for any specified period, and in such case the licence may be renewed from time to time by the Minister, if he thinks fit, for any specified period.

(3) Any licence under this section may be cancelled by the Minister at any time by notice in writing to the holder of the licence.

(Persons having lawful possession of bodies.)

3 (1) Section twenty-four of the principal Act is hereby amended by adding the following subsection as subsection two thereof:

"(2) Without limiting the rights, powers, or duties of any person entitled under any rule of law to the possession of any body, it is hereby declared that for the purposes of this Part of this Act the following persons shall be deemed to be persons lawfully in possession of bodies in the cases hereinafter specified, namely

(a) The Medical Superintendent or other medical officer for the time being in charge of any institution within the meaning of the Hospitals Act 1926, or the licensee of a licensed hospital within the meaning of Part III of that Act, in respect of any body lying in the institution or hospital

(See Reprint of Statutes, Vol III, p. 725.)

"(b) The Medical Superintendent or other medical officer for the time being in charge of any public institution within the meaning of the Mental (Health) Act 1911, in respect of any body lying in the institution being the body of a patient or boarder

(See Reprint of Statutes, Vol. V, p. 743.)

(c) The Superintendent of any penal institution, in respect of any body lying in the institution, being the body of an inmate"

In the United States the usual practice, as for example at the Tissue Bank of the National Naval Medical Center, Bethesda, Maryland, is to obtain written permission from the next of kin before removing tissue from a cadaver (Strong, 1954). Such permission is seldom refused, but it is a distasteful task to thus intrude on the privacy of those who have suffered bereavement. The New Zealand Act, like the British Act but in a more comprehensive way, makes it unnecessary under certain conditions to approach the relatives; at the same time it ensures that nothing is done contrary to their expressed wishes, or to wishes expressed by the deceased before he died. If the full benefit of the Act is to be realized, however, public support must be enlisted, and as many people as possible must be encouraged to make proper legal provision for tissues and organs needed for grafting to be removed from their bodies after death.

Technical Considerations

The tissues now obtained and stored in most banks are as follows: cornea, skin, blood vessels (including aorta), bone and cartilage. It used to be thought essential to remove these tissues within three or four hours of death, but it has now been shown that, provided the body is placed in a refrigerator soon after death, this time may be considerably extended (Deterling, Parshley and Blunt, 1953). For cornea Rycroft (1954) regards the safe upper limit as 15 hours, for arteries (Deterling, 1953) and the other tissues enumerated, 24 hours' delay is permissible (Strong, 1954). Should it become worth while to bank and transplant whole kidneys and endocrine tissues, however, the permissible period of delay will probably be very much less

For artery grafts the donor should preferably be under the age of 35;* satisfactory cornea, skin, bone and cartilage may however often be obtained from quite elderly people, as well as from those dying in middle and early life.

The procedure adopted depends on whether or not the tissues are to be sterilized after being removed from the body by one of the procedures described in Chapter 9

Blood vessel segments are often taken without aseptic precautions and sterilized later. Cartilage and bone, though sometimes stored in antiseptic solutions, are usually obtained aseptically, and with skin and cornea asepsis is essential.

If tissue banking is to be done on a large scale the following facilities should be available:

*Deterling *et al.* (1953) attach more importance to the appearance of the vessels than to the age of the subject, the above is, however, a safe working rule.

1. *A cold room*, maintained at about 4° C, in which all bodies are placed as soon as possible after death.
2. *An operating suite* provided with all the usual facilities for sterilization of equipment and for aseptic surgery.
3. *A work room* which is used for two main purposes: (a) preliminary cleansing before a body is taken to the operating room, and (b) any necessary reconstruction of the body after tissues have been removed.
4. *A tissue processing and storage room* in which procedures such as freeze-drying are carried out and tissues are stored under various conditions.
5. *A records office.*

In addition, the services of a bacteriological laboratory must be available for testing the sterility of samples of tissue.

When tissues are to be removed aseptically, the body is washed with soap and water in the work room and then taken to the operating room. Here a full aseptic ritual is observed. The operator and his assistants wear masks and sterile gowns and gloves. The skin of the body is prepared with merthiolate or some other antiseptic, and sterile drapes are applied as in an ordinary operation. A convenient order of procedure, which is used at the United States Naval Medical Center, Bethesda, Maryland, is as follows (Strong, 1951).

The body is placed face downwards and the following tissues are removed:

1. Sheets of skin from the back.
2. The scapulae.

The body is then turned face upwards and the following structures are removed in the order named:

3. The femoral arteries.
4. The tibiae and femora.
5. The iliac bones.
6. The sternum and selected ribs.
7. The thoracic aorta.
8. The abdominal aorta.

The eyes, if required, may conveniently be removed immediately after the body is turned face upwards.

A number of details of technique require special mention.

Great care must be taken to minimize the risk of bacterial contamination. Whenever the field of operation is changed, all members of the operating team re-scrub and don fresh gowns and gloves. The skin is again prepared and draped, and a new set of instruments is used. Samples of tissue are taken and sent for bacterial culture—anaerobic as well as aerobic—at frequent intervals.

Skin is best removed with an electric dermatome or a Humby knife. A dermatome of the Padgett type, which necessitates the use of adhesive, is not recommended for cutting skin which is to be stored.

In removing arteries, all branches are divided at least a centimetre from the main trunk, as advocated by Strong (1951). The vessels are washed in Ringer's solution to remove any clotted blood.

Bone is removed subperiosteally by a strictly no-touch technique. An electric saw facilitates removal of the long bones.

Tissues as they are removed are placed in sterile containers and stored temporarily in a refrigerator. They may be kept dry or, alternatively, immersed in a balanced saline solution containing penicillin and streptomycin and sometimes homologous plasma, the choice depending on the method of permanent storage which is to be used. The methods of processing and storage which are available have been described in Chapter 9, and will be discussed further in subsequent chapters.

Before grafts are released for use, the bacteriological reports are scrutinized. If any of the tissue samples prove to be infected, all grafts of the tissues in question are discarded.

The following records are essential:

1. A record—best kept in a book—of each body handled by the bank. This includes the name and age of the subject, cause of

death, the results of the Wassermann and other tests, and, in addition, the written authority for the removal of tissues.

2. A card for each graft showing the name of the donor, the date on which the graft was obtained, the method of storage, and the results of bacteriological cultures. When the graft is issued the date of issue, name of the recipient, purpose for which the graft is used, and details of the operation are recorded. Reports on the clinical progress of the recipient are added subsequently.

CHAPTER 13

Skin Grafting and Other Procedures for the Repair of Surface Defects

I. Free Grafts

THE NATURAL REPAIR OF WOUNDS AND AREAS OF SKIN LOSS

Skin loss commonly results from such causes as mechanical injuries, burns, ulceration, and surgical operations undertaken for the removal of tumours, scars and other lesions.

The process of natural healing of wounds of various kinds, with or without loss of skin, has been re-examined in recent years in animals (Abercrombie, Flint and James, 1954; Billingham and Medawar, 1955; Abercrombie, 1956) and also *in man* (Gillman, Penn, Bronks and Roux, 1953, 1955), and in consequence many long established views have had to be modified. The position, in the light of the evidence now available, appears to be as follows.

Incised Wounds

In the healing of incised wounds, according to Gillman *et al.* (1955), the first tissue to regenerate and bridge the gap is the epithelium which always inverts (even when everting sutures are used for closure) and grows into the incision and also into the stitch holes. After closing the defect the epithelium becomes hyperplastic and invasive, and only then, 3-5 days after injury, is there evidence of fibroblastic proliferation, first in the subcutaneous fat, fascia and muscle, and later just beneath the epithelium. As sub-epithelial fibroplasia progresses the epithelium becomes less hyperplastic and its growth appears to be brought under control. Blood and tissue fluid in the wound seem to play no role other than a protective one, and the transected dermis also contributes little or nothing to the process of repair.

Partial thickness Defects

Partial thickness skin defects, such as the donor sites from which split skin grafts (p. 227) have been removed, also become covered by epithelium (as the result of migration and division of cells from the wound edges and from the hair follicles and other skin appendages in the injured area) before there is any evidence of fibroplasia (Gillman *et al.*, 1953, 1955). Four to six days after injury, when epithelialization is complete, an exudate rich in protein and glycogen appears beneath the thin new epithelium and connective tissue regeneration apparently takes place from leucocytes which immigrate into this exudate from the blood stream and from perivascular collections of cells which were *formed at an earlier* stage. After the sub-epithelial regeneration has started *the epithelium becomes hyperplastic* and invasive, and forms pseudo-rete pegs. These are later eliminated by an active connective tissue reaction which is apparently evoked by the penetrating epi-

thelium itself, and eventually the junction of epithelium and connective tissue becomes straight and scar-like (Gillman *et al.*, 1955)

Full-thickness Defects

With full-thickness skin defects the course of events depends on the size of the defect and on the extent to which the skin is normally attached to the subjacent tissue.

When the skin is freely mobile, as is the case with the greater part of the skin in most animals, the healing of full-thickness defects up to a certain size, as Billingham and Medawar (1955) have shown in rabbits, is brought about by two distinct processes *contraction*,* i.e. a forced tissue movement that results in the closure of the wound by the apposition of its original edges, and *intussusceptive growth* (also termed *intercalary growth*), i.e. a true increase in area of the skin brought about by the formation of new tissue upon or within the framework provided by the pre-existing tissue. At the same time granulation tissue is formed within the perimeter of the defect and may be partly converted to fibrous connective tissue and there may be some degree of epithelialization from the margins. Neither the fibroplasia nor the epithelialization makes any *substantive* contribution to the state of final repair (Billingham and Medawar *loc. cit.*) but the former, as Lindquist (1946) has argued, apparently provides the tensile forces that are responsible for the contraction.†

When the defect is large, or occurs in an area where the skin is firmly attached to the underlying tissue, like most of the skin in man and certain areas of animal skin such as the distal ear skin of rabbits and guinea pigs (Billingham and Medawar, *loc. cit.*), contraction and intussusceptive growth may occur but do not suffice to close the defect, and in the absence of skin grafting the end result is a fibrous scar covered by epithelium of migratory origin, or a persistent ulcer.

In either event there ensues permanent distortion of surrounding structures, termed by surgeons *contracture* (*see* footnote * below), and this often results in gross limitation of movement in nearby joints. Except with very small or partial-thickness defects, therefore, early skin replacement is necessary to obtain a good cosmetic and functional result.

When skin loss results from an aseptic operation, replacement should normally be undertaken at once. Similarly, following mechanical injuries the ideal treatment is to replace skin immediately after debridement of the wound; often, however, this is impossible owing to the presence of more serious injuries which demand priority in treatment, or because of sepsis resulting from gross contamination or unavoidable delay in getting the patient to hospital. In patients with skin loss due to burns the first essential is to treat shock; thereafter treatment is designed to control infection and promote the separation of sloughs in preparation for early skin grafting. Spontaneous separation of sloughs usually takes from two to six weeks; the process may, however, be hastened by chemical debridement (Connell and Rousselot, 1951) or preferably the sloughs may be excised.

The process of intussusceptive growth was first demonstrated in rabbits by Billingham and Medawar (*loc. cit.*) by excising a raw

*Billingham and Medawar, and also Abercrombie, speak of *contracture*. This is unfortunate because surgeons use the term contracture to denote the permanent distortion which often results from scar formation.

†It is uncertain whether the pull is caused by shortening of collagen fibres during their maturation or, as Abercrombie (1955) has suggested, by the activity of the cellular components of the granulation tissue. In support of his hypothesis Abercrombie has pointed out that (a) there is no evidence that collagen fibres are contractile under physiological conditions, (b) contraction can proceed normally in scorbutic guinea pigs despite the virtually complete inhibition of fibrillogenesis which accompanies vitamin C deficiency, and (c) contractility, though more highly developed in muscle cells, is probably a property common to many members of the fibroblast family of cells.

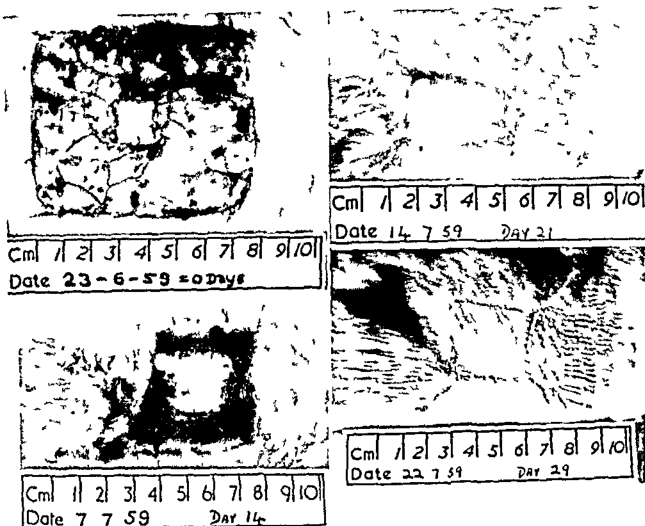


Fig. 55. Intussusceptive growth in an island of skin in a rabbit. The operative procedure was similar to that described by Billingham and Medawar (see text) but the period of observation was only 29 days.

area in such a way as to leave an island of undamaged skin in its centre or by placing an autograft in the centre of a large defect (Figs 55, 56). Both the island and the graft contracted immediately they were released from the pull of the surrounding skin (*v. infra*) but subsequently expanded to several times their original area. After 75-79 days, when contraction of the wound and expansion of the central skin were complete, a new rectangular area of skin was excised in such a way as to leave the original (but now expanded) island in position. This island contracted immediately and continued to decrease in area during the next 10-15 days but thereafter began to expand and continued to do so until it was much larger than it had been even just prior to the second operation.

It seems clear that contraction, as defined above, normally plays little part in the healing of skin defects in man but it may be of importance in special sites, notably the scrotum and the neck. It would be of interest to determine whether intussusceptive growth occurs in human skin grafts when these are applied in the form of strips or "postage stamps" (*see* Figs 62, 63), but so far no accurate measurement of this kind appears to have been made. It would also be of interest to study the process by which the skin overlying an expanding lesion such as a large hernia increases in area.

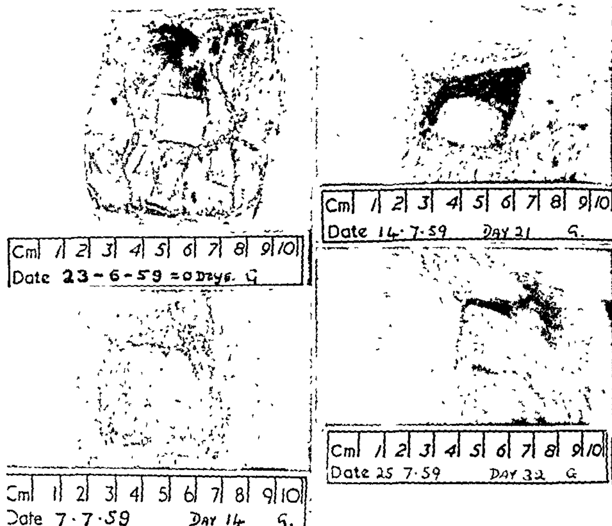


Fig 56 Intussusceptive growth in a free autograft of ear skin placed in a large defect on the side of the thorax in a rabbit. The operative procedure was similar to that described by Billingham and Medawar (see text), but the period of observation was only 32 days.

ELASTIC PROPERTIES OF SKIN

The relative lack of mobility of human skin in relation to underlying structures is due to the fact that except in the neck and the scrotum there is no properly developed subcutaneous muscle corresponding to the panniculus carnosus of animals, and the skin is anchored to the underlying deep fascia by fibrous bands which extend downwards through the subcutaneous fat. These bands are not uniformly distributed, and the skin creases or wrinkles, which are most conspicuous on the face, the neck and the flexor aspects of joints, but which occur also in other sites, indicate the lines along which the skin is most firmly tethered. The creases result from the continual changes in skin tension brought about by the pull of muscles inserted into the skin, or by the movement of nearby joints. Generally speaking, therefore, their direction is, as Rubin (1948) has pointed out, perpendicular to the line of pull of the underlying muscles.

Normal skin is an elastic membrane, and even when the muscles are relaxed it is in a state of static tension which, at any given point, is maximal in one particular direc-

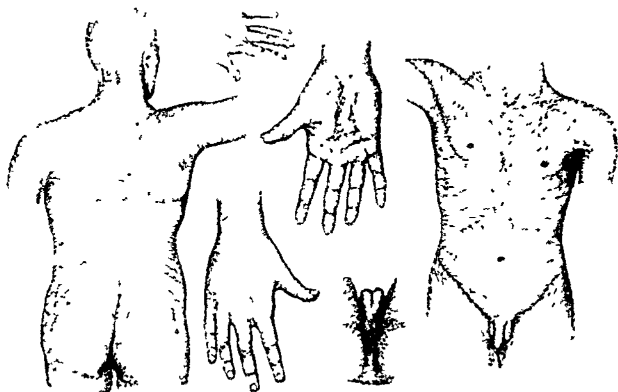


Fig. 57 One of Langer's original charts. (Reproduced from *Sitzungsberichte der Kais. Akademie der Wissenschaften in Wien. Math Naturwiss. Kl.* 1861 by courtesy of the publishers.)

tion. This was first demonstrated with human skin by Dupuytren (1831), who observed that three puncture wounds in the neck of a suicide, which had been made with a round awl, appeared elongated. Dupuytren later showed that the same phenomenon occurred with puncture wounds made *post mortem* in various parts of the body. His work was repeated by Malgaigne (1838), and subsequently by Langer (1861), who made many detailed studies and published charts showing the lines of maximal skin tension everywhere on the body surface (Fig. 57). These lines are today known as Langer's lines. They are often confused with the skin creases, but except in the neck the two sets of lines diverge considerably, and in some regions they intersect at right angles (Fig. 58). This is of surgical importance because in order to avoid producing a conspicuous scar, an incision should be placed in a crease. Langer's lines might be a useful guide if one were concerned to make inconspicuous incisions in a corpse, but, except where they happen to coincide with the skin creases, they should not be taken as the guide for siting elective incisions in living patients (Kraissl, 1951).

The existence of static tension in the skin is shown also by the fact that flaps and free grafts, especially thick grafts, contract when they are freed from the pull of the surrounding skin and from attachments to deeper structures. According to Davis and Kitlowski (1931) the degree of contraction is uniform in all directions, and is independent of the site of the donor area and the age of the patient. Ragnell (1952), however, has cast doubt on these statements.

The elasticity of the skin is due mainly to

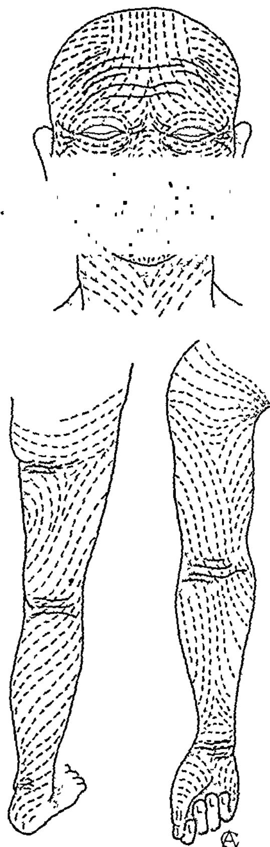


Fig 58. Diagram to illustrate the relationship of Langer's lines (shown as broken lines) to the skin creases (shown as full lines). Note the lack of correspondence between the two sets of lines, especially on the forehead, in front of the elbow and behind the knee

elastic fibres in the dermis. These are most abundant in the outer (papillary) zone round the hair follicles, and in the innermost zone; they are sparse in the middle (reticular) zone.

Quantitatively, elasticity* is measured by the increase in length per unit initial length which is produced by *unit stress*. The stress is the stretching force per unit area of cross-section. The term tension, which in physics denotes the stretching force and not the force per unit area, is often used erroneously in place of stress, as for example when we say that the skin tension at a point is maximal in one direction (*v. supra*).

Langer tried to estimate the elasticity of skin in different directions by studying the behaviour of excised strips under tension, and reported that the elasticity was less in the lines of maximal stress than at right angles to them. To explain his results he postulated that the dermal elastic fibres form a network, the meshes of which are rhomboidal in shape and have their long diagonals in the direction of the stress lines. Ragnell (1952) attempted to repeat these experiments but found that serious errors occurred, due partly to the difficulty of cutting comparable strips and mounting them for testing, and partly to the waist-like narrowing which occurred near the centre of strips subjected to tension. He therefore devised another method in which a circular piece of skin was held at its periphery in a metal ring and stressed by exposing one surface to gas under pressure (Fig. 59). Prior to stretching, concentric circles and equally spaced radiating lines were marked out as shown in Figure 60. Subsequently the distances AO, BO, CO etc. were measured with calipers. In tests with rabbit skin it was found that, up to a pressure equal to five-sixths of that which caused bursting, (AO + OA′) differed from (CO + OC′) by

*This is in accordance with popular usage The *modulus of elasticity* is the reciprocal of the quantity here defined

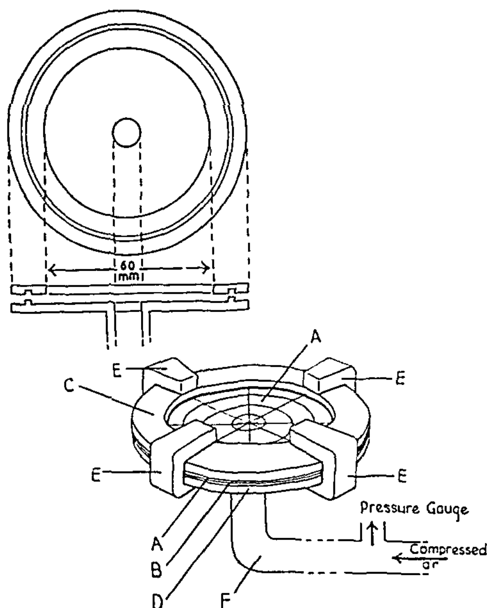


Fig. 59. Ragnell's apparatus for studying the elastic properties of skin. A circular piece of skin (A), backed by a piece of thin rubber (B), is held between a metal plate (C) and a ring (D), which are provided with a circular tongue and groove respectively to prevent slipping, and are fixed together by clamps (E). There is a hole in the centre of (C) connected to a pipe (F) through which compressed air is admitted from a cylinder controlled by a reducing valve.

not more than 1 mm., and all the points returned to their original positions when the pressure was removed. After exposure to slightly higher pressure, however, the skin remained permanently stretched. It was therefore concluded that rabbit skin is equally elastic in all directions and retains its elastic properties under increasing stress until shortly before the bursting point. It is difficult to assess the validity of the first conclusion because, without more detailed information, it is impossible to estimate what difference in elasticity in the two directions AA' and CC' would be necessary to produce a measurable difference in the distances (AO + OA') and (CO + OC'). The method appears promising, however, and it should be of interest to apply it to the study of human skin.

METHODS OF SKIN REPLACEMENT

The methods of skin replacement may be subdivided into two main groups: replacement by free grafts and replacement by flaps. Replacement by flaps is historically much the older procedure but, at any rate as regards flaps from a distance (p. 265), is more complicated and time consuming than replacement by free grafts. It is more appropriate, therefore, to consider free grafting first. Flaps will be dealt with in the next chapter.

HISTORY OF FREE SKIN GRAFTING

The development of free skin grafting dates from the beginning of the nineteenth century when Baronio (1804) and Dieffenbach (1822) began studying experimental grafts in animals.

The first well authenticated case of successful free skin grafting in man appears to be that of Bunger (1822), who used a free full thickness autograft to repair a defect of the nose.

A discovery of decisive importance was made by Reverdin (1870), who showed that a free autograft of human skin could survive *and grow*. Reverdin had observed that the small islets of epithelium which sometimes occurred in wounds gradually increased in size, and it occurred to him that small transplants might behave similarly. He therefore raised two pieces of skin with the point of a scalpel—one about 1 sq. mm. in area, the other even smaller—and transplanted them autologously to a granulating surface; three days later he added a somewhat larger graft. Epithelium grew out and in two weeks the grafts coalesced. Reverdin believed that his grafts consisted only of epidermis, but it is now known to be almost impossible to cut a purely epidermal graft.

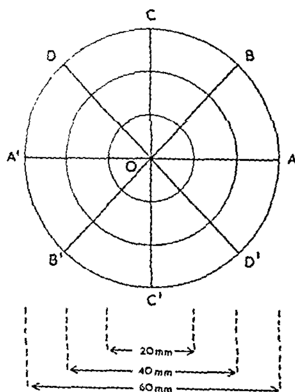


Fig. 60. System of marking used in Ragnell's method of studying the elastic properties of skin. For explanation see text

Reverdin's account of his experiment aroused much scepticism and even ridicule, but his form of graft quickly increased in popularity. At first both autografts and homografts were used without distinction, the latter sometimes being taken from amputated limbs or cadavers. A useful innovation was introduced by Dobson (1870), who treated ulcers by inserting Reverdin type grafts into the granulation tissue. The Reverdin graft evolved finally into the modern "small deep graft" (pinch graft) described by Davis (1927).

Less than a year after Reverdin's demonstration, Lawson (1871) read a paper before the Clinical Society of London in which he described the use of whole-thickness grafts up to half an inch in diameter; he stated that to be successful the graft should consist

of skin only without subcutaneous fat, and should be firmly applied to a healthy granulating surface and maintained there without interruption. Lawson cut his grafts with scissors. He observed that the transplanted skin became vascularized and also acquired sensibility, and he predicted that free skin grafting would revolutionize the treatment of burns and deformities resulting from extensive skin loss.

Le Fort (1872), Wolfe (1875, 1893), Esmarch (1885) and Krause (1893) further developed and popularized the free whole-thickness graft. Krause, for instance, reported 100 cases in which whole thickness grafts up to 150 sq. cm. in area were used to treat chronic ulcers of the leg, and defects of the face following *lupus* or excision of carcinomata. It seems clear, nevertheless, that much of the credit for this type of graft should be given to Lawson.

Ollier (1872) experimented with large grafts up to 8 cm. square consisting of the epidermis and part of the dermis. Subsequently Carl Thiersch (1874, 1886, 1888) devised a method for cutting thin grafts with a razor, and showed that these would often take when whole thickness grafts failed. Esser (1917a) showed that grafts of this kind could be used to repair defects of mucous membranes.

The original Thiersch graft proved to be too thin for most purposes, and a great advance was made when Blair and Brown (1929) introduced a thicker type of graft which they described as a "split skin graft of intermediate thickness." Padgett (1939) and others have invented mechanical devices for cutting large split grafts of uniform thickness (*see* Figs. 61-67).

In recent years a great deal of research has been done, partly in experimental animals and partly in man, on three main topics: (a) new types of graft, including grafts of epidermis and cell suspensions (Najarian, Jackson and McCorkle, 1957), and new methods of fixing grafts *in situ*; (b) skin homografts, and (c) methods of storing skin.

TYPES OF FREE SKIN GRAFT USED IN PRESENT DAY SURGERY

Autografts and homografts are both of value, but the former have a much greater range of usefulness and should be preferred whenever possible.

AUTOGRAFTS

Free skin autografts used in modern surgery are of four kinds: *

1. Split skin grafts.
2. Whole thickness (Wolfe) grafts.
3. Small deep grafts (also known as pinch grafts or Staige Davis grafts).
4. Dermal grafts.

Split-skin Grafts

A split skin graft may be defined as a graft of approximately uniform thickness, removal of which leaves sufficient epithelial elements for regeneration (Brown and McDowell, 1941, 1958). As already stated it is almost impossible to cut a purely epidermal graft. In practice, therefore, split skin grafts consist of the epidermis and part of the dermis, and they range in thickness from about 30 to about 60 per cent of the thickness of the skin (Fig. 61). Re-epithelialization of the donor area is effected by proliferation and migration of epithelial cells in the remaining parts of the hair follicles, and possibly also from sweat gland epithelium.

The usual donor sites are the abdomen, the inner and outer aspects of the thigh, and the inner aspect of the arm, but excellent grafts may also be cut from the chest, back, and buttock, and from the legs below the

* The types of skin graft used in experimental animals are described in Chapter 1.



Fig 65 Cutting a split skin graft with a Humby knife.

from the same site two or three weeks later, and after a further interval it may be possible to obtain a third crop * Re-cropping is of great value in severely burned patients with extensive skin loss Another method, due to Zintel (1945), which may be useful when the available donor areas are insufficient, is to re-split the graft after it has been cut but before it has been removed from the dermatome It is then possible to cover an area twice the size of the original graft if sheets are used, and larger still if the grafts are cut into strips or squares.

It is important to limit blood loss from the donor area by applying hot packs and pressure Robinson (1949), in a study of the blood loss from donor sites in skin grafting operations, found that with care the loss averaged 46 c c. per dermatome drum. The loss was greater with thick grafts than with thin, and with grafts from the back and buttocks than with grafts from the abdomen and chest. The loss was also greater with young, well nourished patients than with older or poorly nourished patients.

*Gillman Penn, Bronks and Roux (1953) have pointed out that the regenerating epithelium covering a split-skin donor site attains its maximum thickness 14-16 days after operation, and have suggested that this should be the optimal time for re cropping Their argument sounds plausible and may well be correct, but there is no real evidence that grafts removed at this stage are better than grafts of skin in a less active state.

Methods of Maintaining Grafts in Position

Split-skin grafts may be maintained in position in the following ways:

1. By dressings only.
2. By sutures.
3. By means of a mould.
4. By the coagulum contact technique.

Dressings. The usual procedure is to use one or two layers of tulle gras, followed by gauze bandage, cotton wool, and either a crepe bandage or elastoplast. The tulle is sticky and adherent at body temperature, and thus helps to prevent the grafts from slipping, but it is virtually non-absorbent and, in view of this and the fact that mineral oils injected subcutaneously or intramuscularly provoke a severe reaction (Nairn and Woodruff, 1955), it is by no means an ideal form of dressing.



Fig 66 Padgett dermatome Adhesive is applied to the drum of the dermatome (A) and to the skin of the donor before cutting the graft (B) The clearance between blade and drum is adjusted to give a graft of the required thickness. The dermatome illustrated is designed for use in adults. A smaller one is available for children

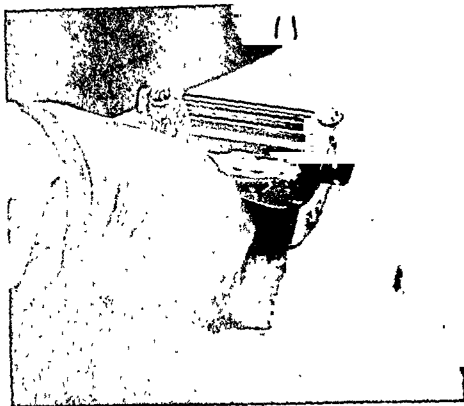


Fig 67 Cutting a split skin graft with the Brown electric dermatome.

Sutures. The grafts may be sutured to the skin at the edges of the defect, and also when necessary to the underlying granulation tissue Fine silk or other non-absorbable material is used, and the ends of some or all of the sutures are left long Pledgets of cotton wool soaked in paraffin-flavine emulsion are applied to the graft and built up to form a pad the surface of which is well above the level of the surrounding skin. The long ends of the sutures are tied over this pad and pressure is then applied by means of a crepe bandage

The dressings are removed and the grafts are inspected after 5-7 days in most cases If the first dressing is performed too early viable grafts may be accidentally detached; if, on the other hand, it is unduly delayed, pus may accumulate from regions where the grafts have failed and cause lysis of the grafts which have taken.

Moulds. Esser (1917a) devised a procedure, which he termed *inlay grafting*, for reconstructing the mouth or conjunctival sac following partial obliteration. He first created an epithelial-lined cavity by introducing, through an external incision, a mould of dental stent surrounded by a split-skin graft with its raw surface outermost; later, at a second operation, he opened this cavity into the mouth (or conjunctival sac) and removed the mould. Subsequently, Pickerill (1918) showed that a skin graft supported by a mould could successfully be applied directly to a defect in the buccal cavity, thus obviating the need for an external incision.

This procedure has proved to be of great value, and nowadays a mould of stent or other plastic material is commonly used to maintain a graft in contact with a concave surface whether the defect is one of mucous membrane or of skin. A remarkable example is the use of a split-skin graft on an acrylic mould for the treatment of congenital absence and obliterative lesions of the

vagina McIndoe (1950) has reported 63 cases in which this operation was performed including 6 with normal uteri who subsequently became pregnant

The Coagulum Contact Technique The coagulum contact method of grafting was devised by Sano (1943a b) and has subsequently been modified by a number of workers It merits more attention than it has so far received from plastic surgeons and will therefore be described in detail

In Sano's original procedure an extract made from the buffy coat* of the patient's blood was applied to the deep surface of the graft and a little of his plasma to the defect When the graft was placed in position the plasma coagulated causing the graft to adhere firmly to its bed A similar technique was used by Sheehan (1944) and by Branch Wilkins and Ross (1946) Harris (1944) obtained equally successful results using homologous plasma and cell extract Tidrick and Warner (1944) simplified the procedure by using homologous stored plasma on the graft and commercial ox thrombin on the bed Young and Favata (1944) and Clark Milne and Todd (1945) used a similar technique except that they applied the *plasma to the bed and thrombin to the graft* Le Veen Franklin and Barberio (1951) in experiments on rats used a one per cent solution of the potassium salt of hyaluronic acid prepared from human umbilical cords by the method of Tolksdorf Cassidy McCready and McCullagh (1950) on both graft and bed They obtained excellent fixation and reported that desquamation, retarded hair growth contraction and prolonged erythema were much less common in grafts treated in this way than in control grafts They suggested that the failure of autografts was sometimes due to the presence of excess hyaluronidase formed by bacteria present on the surface to be grafted and claimed that the deleterious effect of this enzyme could be overcome by fixing the grafts in place with the appropriate substrate, namely hyaluronic acid This is as yet unconfirmed *but the matter warrants fuller investigation* both in man and in experimental animals

* The buffy coat is a layer of white blood cells (leucocytes) which forms between the mass of red cells and the supernatant plasma when blood mixed with an anticoagulant is [illegible]

Free Whole-thickness (Wolfe) Grafts

The indications for using free whole thickness grafts have been discussed in detail by Blair (1921) Gatlock (1933) and, more recently Greeley (1952) Such grafts if freed of subcutaneous fat take well on fresh clean surfaces but are much less likely to survive on granulation tissue

Hynes (1954) has recently put forward a convincing explanation of why full thickness grafts survive less readily than split grafts The deep surface of a full thickness graft appears pitted and Hynes has pointed out that even when macroscopic inspection suggests that all fat has been removed, microscopic examination shows that each pit contains a plug of fat together with the deep part of a hair follicle or a sweat gland or both of these structures This fat impedes first the transfer of tissue fluid to and from the graft and later the process of vascularization The number of pits per unit area *varies according to the donor site* Hynes estimates that with grafts from areas such as the back, the thigh and the extensor and outer aspects of the arm and forearm, where the skin is thick and the pits are numerous about 40 per cent of the area of the deep surface of the graft is ineffective for nutritional exchange With grafts from areas where the skin is thin the pits are sparser and only 10 20 per cent of the graft area is ineffective in this way

Whole thickness grafts should be cut accurately to the size of the defect by means of a pattern and maintained in place by sutures and local pressure Greeley advises

a pressure of 30 mm. mercury applied by means of a pneumatic bag, and he has shown that if the pressure is removed too soon the graft becomes cyanotic.

Full-thickness grafts are especially useful for repairing small defects on the face, the flexor surface of the hands, and the tips of the fingers

Free transplantation of the nipple and areola is sometimes used in reconstruction of the breast (Adams, 1944) Alternatively, pigmented skin from the labia minora is sometimes transplanted to replace areolar skin (Adams, 1949, Spina, 1950).

If hair growth is required, as in reconstruction of the eyebrow by a free graft, a little subcutaneous fat is sometimes deliberately included in order to utilize the full length of the hair follicles, inevitably, however, the proportion of successful grafts is reduced in consequence (Greeley, 1952).

Small Deep Grafts

Small deep grafts are thin at the edges but almost whole thickness in the centre, and are usually about 1 cm. in diameter. They may be cut with scissors or, more easily, by the method shown in Figure 68, and either applied to the raw surface or buried in the granulation tissue. Buried grafts are easier to maintain in position; they do not encyst but gradually find their way to the surface.

Small deep grafts give a poor cosmetic result and leave rather ugly marks at the donor site, but have the advantage that they often take well even in the presence of gross infection.

Dermal Grafts

Buried dermal grafts will be considered with other connective tissue transplants in Chapter 15. We shall, however, consider here a new technique introduced by Hynes

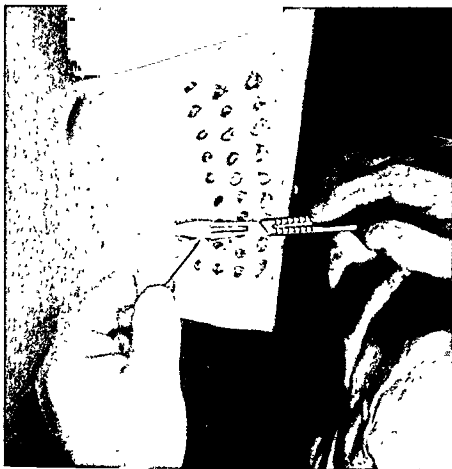


Fig. 68. Cutting small deep grafts.



Fig. [illegible] Treatment of a chronic ulcer of the leg by a skin-dermis graft A The lesion consisting of a small ulcer and an extensive unstable scar B The scar has been excised and the area allowed to granulate C. The reversed dermis graft which in this case was not immediately covered with a split-skin graft D The final result after covering the reversed dermis graft with a split-skin graft (Reproduced from the *British Journal of Plastic Surgery* by courtesy of the publishers and Mr Wilfred Hynes)

(1954) termed *skin-dermis grafting* (Fig. 69). This technique has the great advantage of being applicable to granulating surfaces, and gives excellent functional results superior to those obtainable with split grafts.

The first step* in skin-dermis grafting, assuming the defect has been properly prepared and presents a smooth red granulating surface, is to cut a thin split graft from the thigh, using saline and not petroleum jelly as the lubricant. This graft is put on one side for later use. The dermis thus exposed, together with a thin layer of underlying fat, is then removed by knife dissection, and replaced by a split graft from the opposite thigh. Fat is cut away from the deep surface of the dermis graft with scissors, but not too closely because it is advantageous to leave small scattered areas of fat both in and outside of the dermal pits. The dermis graft so prepared is applied to the granulating defect in a *reversed* position, that is with its superficial surface on the granulating wound and its deep, pitted surface, with its tiny clumps of adherent fat, superficially. The reversed dermis graft is then covered with the thin split-skin graft which was cut from the thigh as the first step in the operation.

The dermis graft takes regularly because the surface it presents to the granulation tissue of the bed is quite free of fat. The thin split skin graft is used primarily as a dressing, it usually does not take and is picked off when the first dressing is carried out on the seventh day. The area is then dressed with saline dressings for three or four days, by which time the dermis graft presents at each "pit" a bright red dot of granulation tissue, the procedure is then completed by covering the dermis graft with a definitive thick split-skin graft. Occasionally, contrary to expectation, the thin "temporary" split-skin graft becomes established; in this event no further operations are necessary.

*The description which follows adheres closely to Hynes' original account of the operative technique.

The dermis-skin graft has proved to be particularly valuable in the treatment of chronic leg ulcers, and can often be used successfully when the only alternative is to use a flap.

The Life History of Free Skin Autografts

Much can be learned of the life history of free skin grafts by repeated clinical examination. The phenomena to be observed include changes in colour due to vascularization or pigmentation, changes in the texture of the skin, changes in surface temperature, recovery of sensation, and, with thick grafts, recovery of the function of sweating.

Further information can be obtained by performing biopsies at intervals after grafting. The observations to be made are of two kinds: (a) the histological features of the specimen, including the mitotic activity in the deeper layers of the epidermis; and (b) the capacity of the epithelial cells of the graft to migrate into the small defect resulting from removal of the specimen.

Finally, skin microscopy (p. 31), and tests such as the fluorescein test and the histamine wheal test (p. 274), may be used.

The life history of a successful free skin autograft may be subdivided into three phases:

1. The phase before re-vascularization.
2. The phase during which re-vascularization occurs.
3. The phase of organic union.

The Phase Before Re-vascularization

Fibrin, formed by coagulation of plasma exuding from the bed, anchors the graft in place, usually within five hours. Nutrition of the graft depends on interchange of fluid between the graft and its bed, a process sometimes termed *plasmatic circulation* (p. 30).

Characteristic morphological changes occur in the graft during the period prior to

re-vascularization. The epithelial cells show degenerative changes and the superficial layers of the epidermis often desquamate; at the same time *the dermis becomes oedematous.* Leucocytic infiltration may be observed between the graft and its bed, in the dermis and *often* also in the epidermis.

The Phase of Re-vascularization

The process of re-vascularization of free grafts has been described in detail in Chapter 2. *It depends partly on the development* of anastomoses between vessels growing from the bed and pre-existing vessels of the graft (Converse and Rapaport, 1956) and partly on invasion of the graft by vessels from the bed. The development of anastomoses may begin within 24 hours of operation and usually ceases by the end of the third day. Invasion by new vessels is a slower process and is not usually complete until about the tenth day.

According to Mir y Mir (1951) the rapidity of vascularization, the relative importance of anastomosis and re-invasion, and *the state of nutrition of the graft during this* phase are affected by the following variables:

1. The thickness of the graft.
2. The degree of tension in the graft.
3. The state of the bed.
4. The pressure applied to the graft.
5. The temperature at which the graft is maintained.

Thickness of the Graft. With very thin grafts the plasmatic circulation is adequate to maintain nutrition for several days, and early vascularization by anastomosis is unimportant. With thick grafts, on the other hand, early vascularization by anastomosis is essential for survival.

Tension. Mir y Mir has found that the maximum tension which may be applied to a graft without impairing its vitality is inversely proportional to the thickness of the graft. A very thin graft, for example, *may safely be sutured under the maximum* tension possible without tearing the graft, whereas with a whole-thickness graft the tension should not exceed that of normal skin. To explain this he suggests that increased tension facilitates the plasmatic circulation, which is important with thin grafts, but at the same time, by distorting the vessels of the graft, it interferes with the process of re-vascularization by anastomosis which we have seen is essential for the survival of thick grafts.

State of the Bed. It is common experience that split-skin grafts will take on all fresh defects other than those exposing tendons *without sheaths or bone devoid of* periosteum. Mir y Mir claims that very thin grafts will take even on bare tendon or bone, especially if part of the graft is in contact with more vascular tissue. Again he bases his explanation on the principle that the plasmatic circulation is able to maintain the nutrition of a thin graft for a long time.

When the bed consists of a granulating surface, a thin graft is much more likely to survive than a thick one. The reason is that every granulating surface, however clean it appears, is swarming with bacteria, and thin grafts are more resistant to infection.

Pressure Applied to the Graft. The pressure *should be sufficient to provide adequate* immobilization and to prevent accumulation of fluid beneath the graft; for this a pressure slightly greater than the *capillary* blood pressure should suffice. In practice, however, strong pressure is unlikely to cause damage except when the graft is thick and there is a hard bony surface subjacent to the defect.

Temperature. A thick graft appears to have a better chance of survival if it is kept cool. The probable explanation is that the metabolism of the graft is retarded by cooling and its nutritional requirements are correspondingly lessened. Ideally, as Mir y

Mir rather wistfully suggests, the graft bed should be warmed to increase the flow of blood while the graft itself is cooled.

Morphological Changes During the Phase of Revascularization. In the epidermis the degenerative changes are gradually arrested and the normal architecture is restored. Cells which have remained viable recover their function and those which have been destroyed are replaced. As Medawar (1946a) has shown there is enhanced mitotic activity in the germinal layer.

In the dermis the formation of new capillaries is associated with fibroblastic proliferation. The elastic fibres usually show little or no change at this stage (Padgett, 1942). The epithelial cells of the hair follicles, sebaceous glands and sweat glands undergo changes similar to those which occur in the epidermis. The layer of fibrin which temporarily fixes the graft to its bed begins to be invaded by fibroblasts about the fourth or fifth day after grafting.

The Phase of Organic Union

By the tenth day there is usually firm fibrous union between the graft and its bed. Degeneration of elastic fibres in the dermis becomes apparent a few weeks after grafting. Subsequently, regeneration of elastic fibres occurs, but the process takes 12-18 months and the new elastic fibres are not arranged in as orderly a manner as those of normal skin (Padgett, 1942). After some weeks a layer of fat begins to develop beneath the graft. The origin of this fat is uncertain and deserves further study. The layer is usually thickest beneath full-thickness grafts, but may be quite marked beneath split grafts even when these have been placed on granulation tissue.

A successful graft remains true to type.* A full-thickness graft from a hair-bearing area, for example, continues to grow hair, and, as Oghi (1950) has shown, despite claims to the contrary a split-skin graft used in reconstruction of the vagina retains the histological characteristics of skin, as distinct from those of vaginal mucosa.

Contraction. Split-skin grafts tend to contract, to an extent which depends on the nature of the underlying tissue and the thickness of the graft. Contraction is minimal when a graft is placed on bone; when, however, the underlying tissues are loose, as in the neck, a thin graft may contract by as much as 60 per cent, and a thick split graft by 10-35 per cent (Padgett, 1942).

With whole-thickness grafts contraction is usually negligible.

Pigmentation. In brunettes, grafts which were initially the same colour as the surrounding skin often become progressively darker, in blonds, on the other hand, grafts, especially thin split grafts, often become unduly pale.

These changes merit detailed study in the light of the modern theory of skin pigmentation, according to which melanin is formed exclusively in pigmented dendritic cells of the epidermal glial system and is subsequently injected, via the branching processes of the dendritic cells, into the cytoplasm of "ordinary" epithelial cells. The phenomenon of pigment spread in guinea pig skin, which is described in Chapter 2, is of interest in this context. It differs from the phenomena under discussion, however, because in the animal experiments black skin was deliberately transplanted to a white area and *vice versa*, whereas in clinical practice the graft and the surrounding skin are usually well-matched at the time of transplantation.

Re-innervation and Recovery of Sensation. The first reliable study of the recovery of sensation in free skin grafts appears to be that of Dubreuilh and Noel (1911), who reported return of sensation within

*See Chapter 2 (p. 29) for an example of this phenomenon in animals.

one year in 11 whole thickness grafts. Williams (1928) examined a Thiersch graft which had been applied to a varicose ulcer thirty years previously. Pain, temperature and deep pressure sensibility were almost normal, but tactile sense was markedly diminished.

Kredel and Evans (1933), Davis and Kitlowski (1934) and Davis (1934) compared the return of sensation in free grafts and flaps. All agreed that it was less complete in free grafts and, according to Davis, occurred in a patchy manner. These findings have not been confirmed, however, and the investigations on which they are based are open to criticism on two grounds. In the first place some of the free grafts had been applied to areas in which nerve endings had probably been permanently destroyed by roentgen irradiation; secondly, the interval between grafting and neurological examination varied from a few months to many years.

A more thorough investigation of the return of sensation in free grafts was carried out by McCarroll (1938), who tested each patient daily for several days after the first dressing was done and then weekly. Pain sensibility was tested by a gravity loaded algesiometer giving a force of 0.7 g., and tactile sensibility was tested with cotton wool. Two-point discrimination was tested in the usual manner. Temperature discrimination was studied in the early cases but proved to be unreliable, probably because heat or cold applied to a graft is transmitted to the underlying tissues. McCarroll's conclusions may be summarized as follows:

1. Complete recovery of sensation can occur in all types of skin graft (free or pedicle) provided that the cutaneous nerves to the defect have not been destroyed.

2. The time taken for return of normal sensation is least with split grafts, intermediate with whole thickness grafts and greatest with flaps. In six out of forty five split grafts tested sensation (including two-point discrimination) was completely normal in three to four weeks, and in a further ten cases it was normal within two months. In the eight full thickness grafts tested the time taken for return of sensation to normal ranged from seven weeks to six months, with a mean of eleven weeks.

3. Pain sensibility returns more rapidly than touch sensibility; the difference, however, is more noticeable with thick grafts than with thin ones.

4. With split grafts, and usually also with whole thickness grafts, recovery of sensation is uniform over the graft provided that the bed is uniform.

5. The state of the bed has a marked effect on the speed and degree of sensory recovery, but the area of the graft makes no difference.

Hutchison, Tough and Wyburn (1949) plotted the pattern of the touch spots in a few free grafts. They concluded that under optimal conditions grafted skin tends to assume the sensory pattern of the skin of the region to which it has been transplanted. More recently Adeymo and Wyburn (1957) have studied the process of re-innervation in skin autografts in rabbits. They found that from the 21st to the 98th day after transplantation the grafts were invaded around their periphery and also on their deep surface by regenerating axons from the cut ends of cutaneous and subcutaneous nerve trunks and by axon branches. The connexion of neuron with end organ appeared to be quite random.

Recovery of Capacity to Sweat. Conway (1939) found that full thickness grafts tested when they had been *in situ* for six months or more were usually capable of sweating, whereas split skin grafts and small deep grafts were not. The absence of sweating in split skin and small deep grafts is clearly due to absence of sweat glands. The presence of sweating in full thickness grafts almost certainly indicates that sympathetic

re-innervation has occurred,* though Conway cautiously refrained from putting this forward as a definite conclusion.

ISOGRAFTS AND HOMOGRAFTS

As we have seen (Chapter 4), skin grafts exchanged between identical twins behave like autografts, and having once taken survive permanently (Bauer, 1927; Brown, 1937; Schattner, 1944; Converse and Duchet, 1947; McIndoe and Franceschetti, 1950). Grafts exchanged between non-identical twins showing blood chimerism also survive for a long time but it remains to be seen whether they are eventually replaced by host tissue (p. 120) Moreover in about 25 per cent of cases homografts from mother to child, and occasionally homografts from child to mother, survive for periods ranging from a month to more than a year (Peer, 1957; Peer, Bernhard and Walker, 1958; Peer and Walker, 1958).

Homografts between unrelated individuals, or between relatives apart from the exceptional cases specified above, take almost as readily as autografts and become vascularized in the same way (McGregor, 1955, Converse and Rapaport, 1956), but are usually destroyed within one to four weeks, and very rarely survive longer than ten weeks (Lexer, 1919, Holman, 1924; McWilliams, 1924; Coller, 1925; Padgett, 1932; Gibson and Medawar, 1943; Rogers, 1950; Woodruff, 1952a, b, Bishop, 1955; Rogers and Allen, 1955a, b: Snyderman, Rogers and Allen, 1956; Peer, 1957; and many others).

Reports of permanent survival require critical scrutiny Many of them are unconvincing because the patients were not under continuous observation for sufficiently long periods after grafting, and it seems likely that temporary survival, combined with ingrowth of host epithelium beneath the graft from the edges of the defect, was mistaken for permanent survival. Some reports however, like those of Davis (1909, 1927, 1944) and Wolf (1946), appear to be well authenticated, and in the author's view the now prevalent belief that skin homografts *never* survive permanently (unless the donor and host are identical twins, non-identical twin chimeras, mother and child or *vice versa*) is unjustified. The observation of McCoy (1949) that claims of permanent survival were much more common forty or fifty years ago, when most surgeons assumed that homografts and autografts behaved similarly, does not necessarily imply that all these claims were false; it is conceivable, as Rogers (1950) has suggested, that the number of permanently surviving grafts was actually greater in those days simply because homografting was undertaken so much more frequently.

*See Chapter 14 (p 277).

Attempts to Obtain Long-term Survival

Attempts have been made to obtain long-term survival of human skin homografts by choosing as donor a member of the same blood group as the recipient, or a close relative, by using several donors for one recipient, by using foetuses and new-born infants as donors; by using purely epidermal grafts; by various procedures designed to inhibit the homograft reaction; and by injecting the prospective recipient soon after birth with cells from the prospective donor.

Attempts to Match Donor and Host by Blood Typing

Even though, as has been suggested, skin homografts may occasionally survive permanently, it is clear that the probability of this happening when the donor is chosen at random is so small as to be, clinically speaking, negligible If, however, a simple test could be devised by which one could determine in advance whether or not a homograft from a particular donor to a particular recipient would survive, it might be possible, by testing a sufficiently large number of donors, to select an appropriate one in any given case. It might even be possible to subdivide

human beings into skin groups such that grafts would survive permanently when interchanged between members of the same group but not when interchanged between members of different groups.

Can blood grouping be used for this purpose?

Contrary to the belief of many of the earlier workers in this field (Masson 1918, Shawan 1919, Neuhof and Hirshfeld 1923, Dobrzaniecki 1929, and others) it is now well established that homografts between *members of the same ABO blood group do not normally survive* for long periods (Holman 1924, Coller 1925, Davis 1927, Brown 1937, Binhold 1939, and others). It would be a formidable task to amass sufficient observations to determine whether there is *any* significant difference between the mean period of survival of grafts interchanged between randomly chosen members of the same group and those interchanged between individuals chosen at random from the general population, but there is almost certainly no gross difference.

Investigations in which the donor and host were identical with respect to the Rh and MN systems, besides belonging to the same ABO group, have been less numerous. For the most part the life of the grafts was not prolonged (Rogers 1950), though Spieth and Capriotti (1948) claim to have obtained permanent survival in one instance.

Woodruff and Allan (1953), taking advantage of recent developments in blood typing, carried the matter a stage further and interchanged small full thickness skin grafts between two volunteers whose blood group combinations were both as follows: O, cde/cde, MN, S negative, Kell negative, Lewis (a) negative (b) positive, Lutheran negative, Duffy positive. In addition both gave a negative indirect Coombs test with each other's red cells as well as with known Rh positive cells. The homografts both showed a good initial take but, in spite of the remarkable degree of red cell compatibility, were destroyed within three weeks; control autografts on the other hand survived permanently. It was therefore concluded that identity of red cell antigens in donor and host, at least to the extent specified, is not a sufficient condition for the survival of *homografts* of skin. It seems unlikely, moreover, in view of the short period of survival in this experiment, that further refinements in red cell grouping will provide a solution to the problem.

There remains another line of approach which is as yet largely unexplored, namely to try to develop methods of typing based *on the antigenic properties of leucocytes*.

If skin groups do exist there is likely to be a very large number of them. It is conceivable, however, that some skin groups, like some red-cell groups, might be common and others rare, in which case the chances of any two people chosen at random belonging to the same group would be much *greater than the reciprocal* of the number of groups. Moreover, *the probability* of obtaining permanent survival of a homograft made in one direction from a randomly chosen donor to a randomly chosen host is likely to be much greater than the probability that two randomly chosen people should belong to the same skin group, that is to say *that grafts could be made successfully from one to the other and vice versa.* For according to the acquired immunity hypothesis (Chapter 5) the *necessary* and *sufficient* condition (assuming a satisfactory grafting technique) for the permanent survival of a graft from a donor X to a recipient Y is that X's skin should contain no antigens which are absent from Y's, whereas the necessary and sufficient condition that reciprocal grafts interchanged between X and Y should both survive permanently is more stringent, namely that X's skin should be antigenically identical with Y's.

Use of a Close Relative as Donor

Occasionally, as in the case described by Converse and Duchet (1947), an identical twin is available as a donor, and his skin can be used to provide permanent repair of a large defect when there is not sufficient autologous skin available. A non-identical twin is only likely to be better than an unrelated donor if the pair show blood chimerism (p. 119) and the chances of this are so remote that they can for all practical purposes be neglected.

Failing an identical twin the patient's mother, or, in the case of a patient who is a mother, her son, is likely to be the best donor (p. 129).

Skin grafts from other relatives do not appear to survive any longer on average than those from randomly chosen donors.

Multiple Donors

The rationale of using multiple donors rests on two assumptions which Medawar (1944, 1945) has shown to be true in the rabbit, and which probably hold good also in man.

1. The speed with which immunity develops depends on the quantity of skin transplanted, so that, other things being equal, a small homograft survives longer than a large one.

2. While a homograft may render the recipient more resistant to homologous skin from members of the donor species in general, the increase in resistance to skin from other donors is much less marked than that to skin from the original donor.

It follows that, if grafts from several donors are transplanted to one recipient, the resistance to a given graft is the sum of a large component induced by itself and a series of small components induced by the other grafts, it is thus less than the resistance which would have been induced by covering the whole area with skin from the donor of this particular graft.

Dempster and Lennox (1951) have reported encouraging results with multiple donors in experimental animals; and McCoy (1949), Sanders and Moore (1950), Mowlem (1952), and others have used the method clinically.

Foetal and Immature Donors

Homografts of foetal skin were tried by Lexer (1914). They took and enlarged rapidly, but were cast off in the third week. More recently Helsingen and Helsingen (1957) have used homografts of skin from a premature still-born infant in one case. Again the grafts took well, but they survived for only seven weeks. Barker (1947b), as we have already noted (Chapter 4), has obtained similar results in animals. Sachs and Goldberg (1943), on the other hand, have reported successful homotransplantation of skin from premature infants, but they do not give sufficient details for the value of their findings to be assessed.

Homografts of preputial skin from new born babies have been used by Lucas (1884), Eisenberg (1919), Esser (1922, 1944), McNealy (1933), Ashley (1937), and Sachs and Goldberg (1943).

To cover large areas multiple donors had to be used, since each donor could contribute only one foreskin. This in itself, as we have seen, is conducive to long survival, and to make a valid comparison with homografts of adult skin the number of donors used for each recipient, and the amount of skin contributed by each donor, would have to be the same for both types of graft. No planned investigation of this kind has been undertaken, and such data as have been obtained are too fragmentary to allow a definite conclusion to be drawn. In many instances the period of observation has not been stated, in others, as in Lucas' case, it was too short for the findings to have any significance. Sachs and Goldberg reported that in one patient most of the grafted skin was still present after two years, and that in some others grafts could be recognized eight

months after operation, unfortunately, however, the number of patients examined at this stage, and the proportion showing persisting grafts, was not stated. On the facts available, therefore, the claim that grafts from immature donors, whether foetal or new born, survive on average longer than comparable grafts of adult skin must be regarded as unproven.

Epidermal Grafts

Rogers (1950) and Pickerill (1951, 1952) have suggested that epidermal homografts might survive permanently, and Pickerill has gone so far as to suggest the possibility of establishing epidermal skin banks. As far as the author is aware no one has published a report on epidermal homografts in man, but the experiments of Billingham and Sparrow (1951) in animals, which were considered in Chapter 1 (p. 52 footnote), make this hope appear very unlikely of fulfilment. Moreover, even if epidermal grafts could be made to survive it seems very doubtful whether they would be sufficiently robust ever to be exposed without dressings, and they would almost certainly be less effective than conventional grafts in preventing contracture.

Procedures Designed to Inhibit the Homograft Reaction

Some of the procedures which have been used experimentally to inhibit or delay the homograft reaction, such as reticulo-endothelial blockade and total body irradiation, are unsuitable for clinical use with skin homografts because the danger is out of all proportion to the possible benefit to the patient. Others, however, including administration of cortisone, ACTH, and antihistamine drugs, have been tried clinically, and it will be convenient at this point to summarize the results obtained. The rationale of these procedures, and the relevant animal experiments, have been discussed in Chapter 6.

Administration of Cortisone and ACTH. As mentioned previously (Chapter 6), it was at first reported that administration of cortisone and ACTH greatly prolonged the life of skin homografts in man, and in some cases resulted in permanent survival (Whitelaw, 1951). Further work, however, failed to confirm the earlier findings (Baxter, Schiller, Whiteside, Lipshutz and Straith, 1951, Ellison, Martin, Williams, Clatworthy, Hamwi and Zollinger, 1951, May, Oakey and Pilling, 1952), and although the possibility of some slight effect cannot be excluded it is now clear that neither cortisone nor ACTH, within the limits of tolerance, prolongs the period of survival to an extent sufficient to be of clinical importance.

Systemic administration of these hormones in large dosage is likely to cause sodium retention, hypertension and other unpleasant sequelae. In view of this, and the observation of Billingham, Krohn and Medawar (1951b), and Woodruff and Llaurado (1955, 1956), that repeated local application of corticosteroids prolongs the life of skin homografts in the rabbit, it seemed worth while to study the effect of applying cortisone locally to homografts in man. The results, however, have been entirely negative (Woodruff, unpublished).

Administration of Antihistamine Drugs. Foster and Hanrahan (1948) administered *pyribenzamine* for sixty days to a coloured female who had received a split skin homograft from a white male. The graft was observed for ninety days and appeared healthy throughout this period.

The procedure warrants further trial in man, although experiments in animals have been discouraging (Woodruff and Boswell, 1954).

Attempts to Induce Specific Immunological Tolerance

The author's attempts to induce specific immunological tolerance to the tissues of a particular donor by injecting human infants

at birth with leucocytes from the donor in question have been discussed in Chapter 7. For the present, as we have seen, it has been deemed wise to abandon these investigations pending further study of the graft against host reaction in animals, but if some modification of this procedure can be devised which is both effective and safe it may become of great clinical importance

Indications for Using Skin Homografts

Homografts, even though they survive for only a few weeks, may nevertheless be of great clinical value in patients with extensive skin loss due to burns (*see* Fig 42) or mechanical injuries, and may sometimes fairly be described as life saving (Bettmen, 1938 McCoy, 1949, Woodruff, 1952a, b, 1957 1959, Jackson, 1954, Brown and McDowell, 1942, 1958), and the same is true of freeze dried homografts which provide excellent temporary cover (*see* Fig 51) but are dead and therefore cannot really be said to survive at all By using alternate strips of autologous and homologous split skin grafts, as recommended by Mowlem (1951, 1952), large defects may be covered at one operation If several donors are used most of the homografts will usually survive for several weeks, and those which do can be replaced by fresh autografts cropped from the original donor areas (Woodruff 1952a, b) In the meantime the patient's general condition has had time to improve infection has been controlled, and the formation of scar tissue has been reduced to a minimum Alternatively, if the homograft strips are not more than ½ in wide, they may be gradually replaced by epithelium from the adjacent autografts and the need for further grafting may be avoided (Jackson, 1954).

At the present time homografts are usually applied after the sloughs have separated, but it would greatly hasten recovery if the sloughs could be excised within a day or two of burning or even earlier and the whole defect completely covered with a mixture of autografts and homografts. One difficulty is that the operation would add further trauma, but with careful control of fluid and electrolyte intake, including administration of adequate amounts of whole blood, the problem of shock should not be insoluble. Another reason why this procedure is seldom attempted is that it is extremely difficult to determine the precise limits of deep (i e full-thickness) burning. Recently however Lewis (1958) has reported that this can be done accurately by giving the patient an intravenous injection of 20 ml. of 10 per cent disulphine blue and examining the burned area five minutes lat[illegible]

[illegible] burns

s[illegible] even more intense blue colour, and deeper partial thickness burns show either green stippling on a green background or green stippling on a red background, whereas areas of full thickness of burning are not coloured by the dye at all. If this work is confirmed early excision of even extensive deep burns may well become standard practice, for if the patient is not fit to withstand the cutting of autografts the defect can be covered for the time being entirely with homografts It is to be hoped however that some other dye will be found which will be equally effective without dyeing the whole patient even temporarily.

Homografts may also be of value in the treatment of exomphalos. In this condition it is often impossible to replace the gut in the abdomen at the primary operation, and the hernia must be covered temporarily with skin to prevent rupture of the sac. Local flaps are usually employed for this purpose, but if they cannot be approximated without excessive tension, or if the wound subsequently breaks down, free grafts must be used. In these circumstances the use of a homograft from one of the parents, as described by Welch (1951), may again be a life-saving measure.

HETEROGRAFTS

Skin from all sorts of animals including

1919) but this practice has long since been abandoned

In recent years however the possibility of using calf embryo skin in badly burned patients—including if they should occur atomic warfare casualties—has been raised by Silvetti This material was first used by Silvetti and Fernandez in 1955 (cited Silvetti *Cotton Byrne Berrian and Menendez* 1957) in a 72 year old woman with a large full thickness skin defect measuring 20 × 9 cm On the 16th post-operative day the graft had almost completely disintegrated but the defect subsequently healed in about five weeks

Further clinical trial of skin from 2½ to 4 months old calf embryos has been undertaken in human volunteers with partial thickness skin defects and in a few patients with small full thickness defects by Rogers Converse and Silvetti (1957) In no case were any untoward effects observed The period during which the grafts provided effective cover ranged from 12 to 17 days

STORAGE OF SKIN GRAFTS

Human skin may be stored for future use as either an autograft or a homograft

Storage for autografting is indicated especially when owing to variation in the time required for sloughs to separate or for infection to be brought under control the areas to be grafted are not all ready at the same time This often happens in burned patients By cutting all the skin required at one sitting it may be possible to complete the whole procedure with only one anaesthetic because the stored skin can be applied without anaesthesia as a dressing when required Matthews (1945) recommends that if this procedure is followed the grafts should be fixed in place by the *coagulum* contact method (p 235)

It may also pay to cut and store a little spare skin even when the defect is covered in one operation to allow for possible failure of part of the graft

It has been suggested that on granulating surfaces stored autografts may yield a higher proportion of takes than fresh ones (Matthews 1945 Flatt 1950) There is however no definite evidence to support this Matthews has been careful to emphasize that he did not have enough cases for his findings to be statistically significant and Flatt bases his claim on the rather unconvincing observation that stored grafts often survived *in spite of a degree of infection* which is usually assumed to be too great for normal successful fresh skin grafting

Stored skin for homografting should be available in every large hospital Its main value is that it can be used to provide temporary cover in patients with such extensive skin loss that repair in one operation by autografting is impossible In addition stored homografts may usefully be applied preparatory to autografting with either free grafts or flaps when owing to the presence of infection or other causes it appears uncertain whether an immediate autograft would survive In such cases if the homograft takes it may confidently be replaced later by an autograft while if it fails no harm has been done

It is conceivable that methods of storage might be developed which would modify the skin in such a way that it would survive longer after homografting than fresh skin but so far this has not been achieved If by any means homografts could be made to survive permanently the demand for stored skin would obviously be increased enormously

Methods of Storage

Methods of storage, and the history of their development, have been discussed fully in Chapter 9. Here we shall consider only those methods which are most generally useful for storing human skin for clinical use.

Short-term Storage

When skin has to be kept for not more than about three weeks,* it is satisfactory and convenient to store it at ice-box temperatures. This method was first used by Wentscher (1903).

The skin may be wrapped in phofilm (Webster, 1944a, b), saline-moistened gauze surmounted by tulle gras (Matthews, 1945), or tulle gras alone (Flatt, 1948, 1950), and then placed in a tightly stoppered bottle and stored in an ordinary refrigerator at 1° to 6 C. Alternatively, the skin may be stored in a serum-saline medium as advocated by Hanks and Wallace (1949), Marrangoni (1950), and others.

The author prefers Matthews' method. Storage in a serum-saline medium takes up more room and increases the risk of contamination. It gives no better results than the simpler methods when the period of storage is not greater than three weeks, and is unsatisfactory for very prolonged storage.

*Skin may remain viable at ice box temperature for 8 weeks (Matthews 1945) or even longer (Marrangoni, 1950) but it is unwise to assume that it will do so.

Long-term Storage

For long-term storage by far the best method is to soak the skin in 15 per cent glycerol in saline for half an hour, blot it between layers of sterile gauze, freeze it slowly in a sterile pyrex tube, and store the tube in a mass of solid carbon dioxide. Billingham and Medawar (1952), as we have seen, found that this method enabled rabbit skin to be stored for a year or more, and the author (Woodruff, 1959) has found that it gives excellent results with human skin (*see* Figs. 42, 43).

Freezing without using glycerol (or some substance having a similar action) gives much less satisfactory results, and, in the author's view, should be abandoned as a means of storing skin.

Freeze-drying has been used extensively by Strong and his colleagues (Strong, Turner and Bassett, 1953; Strong, 1954) for storing human skin. It has the great disadvantage, compared with the glycerol-freezing method, that the grafts cease to be viable. In our present state of knowledge this may not matter very much, but if it ever becomes possible to make skin homografts survive permanently it will obviously be essential to use viable grafts.

TRANSPLANTATION OF MUCOUS MEMBRANE

Free transplantation of mucous membrane is of very limited practical value because, if free grafts are required to close a mucosal defect, it is usually preferable to use skin. Hughes (1946), however, has successfully treated alkali burns of the eye by excising the damaged conjunctiva and episcleral tissue, and applying free grafts of oral mucosa or of conjunctiva from the opposite eye, and more recently Kiehn (1954) has used oral mucosa for repairing defects of the conjunctiva and for reconstruction of the urethra, and Marshall and Spellman (1957) have used bladder mucosa for reconstruction of the urethra.

THE USE OF HUMAN AMNION IN SURGERY

Many years ago Sabella (1913) and Stern (1913) reported cases in which defects due to burns, and intractable chronic leg ulcers, healed following the application of fresh human amnion. The part of the amnion which covers the umbilical cord appeared to be especially effective; it was applied with its inner (cord) surface—to which a certain amount of Wharton's jelly remained adherent—in contact with the floor of the ulcer.

Amnioplastin, that is amnion which has been cleaned, soaked in alcohol and then boiled in distilled water, was introduced by Penfield and his colleagues (Chao, Humphreys and Penfield, 1940) for repairing dural defects resulting from compound fractures of the skull, and for preventing adhesions between the brain and the dura after craniotomy. According to these workers the material is absorbed in about 30 days, but by this time defects in the leptomeninges and the dura have healed, and adhesions to the brain are minimal. Following this work amnioplastin enjoyed a considerable vogue in neurosurgery, but to-day most surgeons make no attempt to repair the dura after aseptic craniotomy, and they repair defects associated with compound fractures of the skull with free grafts of fascia lata or pericranium, or with polyethylene sheet. If amnioplastin is used it should be prepared as originally described. In particular, as Penfield (1940) has emphasized, it must be boiled; if unboiled amnion is used dense adhesions may develop.

Various attempts have been made to prevent adhesions in other sites by applying amnioplastin, notably, after laparotomy, and after operations on peripheral nerves (Rogers, 1941) and tendons (Pinkerton, 1942). Some good results have been reported but the method does not appear to have stood the test of time. Law and Philip (1941) have advocated the use of amnioplastin for the treatment of defects of the conjunctiva, and have reported one case.

Troensegaard Hansen (1950), impressed by the earlier work of Sabella and Stern with fresh amnion, has used amnioplastin in the treatment of chronic ulcers of the leg. The membrane was applied with its smooth surface (that is, the surface normally in contact with the amniotic fluid) in contact with the ulcer floor. Following the application pressure dressings were applied and the patient was kept in bed for ten weeks. At the end of this time there was marked ingrowth of epithelium, and any uncovered granulation tissue which remained appeared healthy. The amnion over the surface of the ulcer had disappeared—presumably it was digested by enzymes present in the exudate—but that around the periphery remained.

In trying to assess these findings it must be remembered that most chronic ulcers will heal, at least temporarily, if the patient is kept in bed for ten weeks. One patient treated by the author, for example, had almost symmetrical bilateral post-thrombotic ulcers and both were healed after two months' rest in bed though amnion was applied only to one. Claims that amnion promotes healing must therefore be regarded somewhat sceptically, but it would be wrong to dismiss them without further investigation. If it could be shown that amnion facilitated the ingrowth of epithelium it would remain to determine its mode of action. This might conceivably be purely mechanical, or it might depend on the liberation of some growth-stimulating substance.

More recently Troensegaard Hansen (1956) has used transplants of amnion as a form of tissue therapy (*see* Cordero, 1957; Rogers, 1957) for the treatment of obliterative arterial disease. Improvement has been reported in patients complaining of intermittent claudication treated with this mate-

rial and also with transplants of placental tissue (Le Grand, 1956; Falconer and Gunn, 1959), though it has almost always been subjective and unaccompanied by demonstrable objective improvement in the nutrition of the skin, the oscillometric readings or the arteriographic findings. Falconer and Gunn have suggested that the amelioration of symptoms may have been due to oestrogen or some other hormone liberated by the transplant

Skin Grafting and Other Procedures for the Repair of Surface Defects

II. Flaps

A flap or pedicle graft is a portion of skin and subcutaneous fat which has been raised from the underlying tissue but which remains, at least for a time, connected at some part of its periphery to the donor site by a pedicle containing blood vessels.

Only autologous flaps should be used clinically. Parabiotic flaps have been tried but so far they have proved both useless and dangerous.

Autologous flaps are of two kinds: *local flaps* derived from the tissue in the immediate vicinity of the defect, and *flaps from a distance*. When flaps from a distance other than island flaps (p 270) are used the pedicle is severed after the flap has acquired an adequate blood supply by ingrowth of vessels at the new site. When local flaps are used the need for dividing the pedicle does not arise except in the special case of transposition flaps (p 255).

HISTORY OF FLAP GRAFTING

Skin flaps from the forehead and cheeks were used by Indian surgeons more than 2000 years ago, principally in the operation of rhinoplasty (Figs 70, 71), and the operation is described in the Sushruta Samhita *(Kaviraj Kunjalal Bhishagratna 1907-1916. See also Withington 1894, Garrison 1929, Koch 1941, Maltz 1946).* The use of flaps formed from tissues in the vicinity of the defect for repairing lesions of the ears, nose and lips was also described by Celsus *(see Spencer 1935)* and other Graeco-Roman writers of the first century A.D. Thereafter no new developments occurred until the fifteenth century, when Italian surgeons devised a new method of rhinoplasty employing a pedicle flap from the patient's arm. This operation (Fig 72) was subsequently perfected and widely used by the celebrated Gasparo Tagliacozzi of Bologna (1597). Tagliacozzi also suggested using a pedicle flap from another person but abandoned the idea as impracticable. Unfortunately, rhinoplasty was violently opposed by both surgeons, including Fallopius and Ambroise Paré, and by the Church, which condemned the operation as interfering with the handiwork of God. Tagliacozzi's remains were exhumed shortly after his death and reburied in unconsecrated ground, and for nearly 300 years plastic surgery fell into disrepute and disuse *(see Gnudi and Webster, 1950).*

In 1794 two English surgeons stationed in India, Thomas Cruso and James Findlay, witnessed an operation for rhinoplasty performed by a contemporary Indian surgeon

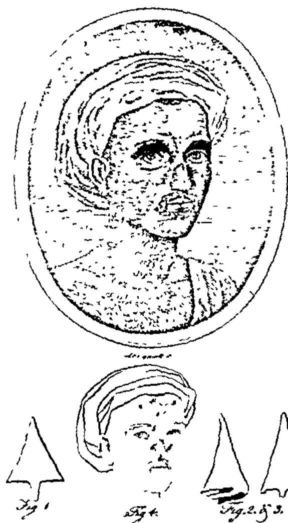


Fig 70 Rhinoplasty performed by an eighteenth century Indian surgeon, according to the ancient Hindu method (Reproduced from the *Gentleman's Magazine* 1794)

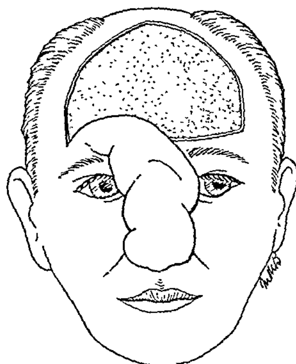


Fig 71 Modern form of Indian rhinoplasty.

according to the ancient Hindu method, and an account of the procedure was given in a letter (signed B L.) to the *Gentleman's Magazine* Interest in flap grafting revived, and during the nineteenth century the procedure became firmly re-established, largely owing to the work of Carpue, Travers, Liston and Whirter in Britain, Lisfranc in France, Dieffenbach and von Graefe in Germany and Mutter, Pancoast and Warren in America The development of anaesthesia and antiseptic methods greatly enhanced the scope of this, as of every other, branch of surgery.

The use of staged transfer was reported by Halsted (1896), who described the process as "waltzing of flaps."

During the first World War plastic surgery received a great impetus, one of the most important developments being the tubed pedicle graft (*see* Fig. 85). The brilliant work of Gillies, and his associates Newland, Waldron and Pickerill (*see* Gillies, 1920a, b and c, 1939) established the importance of this type of graft, but priority of discovery must be accorded to Filatow (1917, 1922), who made the first tubed pedicle graft and used it to reconstruct a lower eyelid.

Scarcely less important was the discovery made by Perthes (1917), and subsequently developed by Blair (1921), that the risk of necrosis was diminished if a flap was "delayed," that is, fashioned in two or more staged operations.



A



B



C



D

Fig. 72. Italian form of rhinoplasty as performed by Tagliacozzi. A. Arm flaps raised but left attached by a pedicle at each end, illustrating Tagliacozzi's use of the principle of delay. B. The pedicle at one end of the flap has been divided. C. Arm flap attached to nose. D. The pedicle has been divided, releasing the arm; bandages have been applied to improve the shape of the reconstructed nose. (Reproduced from Tagliacozzi's *De Curtorum Chirurgia per Insitionem*, 1597.)

INDICATIONS FOR FLAP GRAFTING

Repair by flaps is usually much more complicated and time-consuming than repair by free grafts, especially when flaps from a distance are used; it should, therefore, be undertaken only when it is really necessary. The main indications for using flaps are as follows:

1. When it is necessary to transplant subcutaneous fat as well as skin, for example, to cover exposed tendons.
2. When there is a defect of both mucous membrane and skin, and there is no solid bone against which pressure can be made.
3. When skin replacement is to be followed by operation on bones, tendons or nerves.
4. For repairing weight bearing areas, notably the sole of the foot.
5. When it is impossible to prepare a bed capable of providing quickly for the nutrition of a free graft.

GENERAL PRINCIPLES GOVERNING THE USE OF FLAPS

In reconstructive operations in which flaps are used there are five principles of paramount importance which must be observed.

Firstly, the flap must fit into the defect accurately without undue tension.

When a flap is raised it contracts because it is freed from the pull of the surrounding skin and from attachment to deeper structures. It may be restored to its original size by restoring the original tension; moreover, owing to the great elasticity of skin when raised from the underlying tissues, it may be moderately over stretched at the cost of only a slight increase in tension. If it is greatly over stretched, however, the tension becomes excessive, with the result that neighbouring structures are distorted, sutures may cut out, scars tend to spread, and the blood supply of the flap may be imperilled.

Flaps should therefore be sutured without tension, or, if this is impossible, under tension which is equal to or only slightly greater than the normal skin tension. An exception is sometimes made when a lesion is excised in stages and replaced by a local flap (p. 255). This is permissible, provided that the skin is not stretched beyond its elastic limit and is securely anchored to bony points to prevent distortion, because the elastic fibres gradually yield without losing their elasticity, and, as Ferris Smith (1951) has shown, after about six weeks the tension has fallen to normal and the flap may be further advanced.

Secondly, the flap must at all times have an adequate blood supply.

Raising a flap necessarily involves division of blood vessels. If the resulting diminution in blood supply is not sufficient to jeopardize survival the flap may be fashioned and placed in its new position in one operation, otherwise it must be "delayed." The rationale of delay, and other methods used to improve the circulation in flaps, will be discussed later (pp. 268, 276).

Thirdly, infection must be kept to a minimum.

This is achieved by careful asepsis, by applying protective dressings, and when necessary, by administering antibiotics.

Fourthly, the parts must be effectively immobilized.

When local flaps are used immobilization sometimes presents no problem. In other cases, however, the parts must be immobilized by means of adhesive strapping or plaster of paris. If plaster is used it is sometimes advantageous to apply the casts a day or two

before the operation and cut windows to expose the defect and the donor area. The operation can then be performed through the windows, after which it is a simple matter to fix the casts together with more plaster.

Fifthly, any secondary defect created in the donor area must be repaired.

It is often sufficient to undermine the skin edges and suture them together; sometimes, however, a free skin graft must be used.

LOCAL FLAPS

Local flaps are formed from tissues in the immediate vicinity of the defect and thus, as von Deilen (1951) has emphasized, provide skin of appropriate colour, thickness and texture.

Various types of local flap are described below. They are often fashioned and transferred in one operation, but must occasionally be delayed.

Sliding, Advancement and Rotation Flaps

Owing to the great elasticity of skin it is sometimes possible to close a defect with a local flap without creating a secondary defect elsewhere. Flaps used in this way are classified according to their geometrical form as sliding, simple advancement, VY and YV advancement, rotation, and rotation advancement flaps (Fig. 73). They are used mainly for closing small defects, but may also be used to provide skin coverage when a large lesion is excised in stages. Staged excision was first described by Morestin (1915a, 1916) and later popularized by Davis (1929), but, as practised by these surgeons, was of limited application because they were reluctant to undercut the surrounding skin. The credit for combining staged excision with repair by local flaps belongs to Ferris Smith (1950, 1951), who used the method widely for treating extensive scars, haemangiomata and other benign lesions, especially on the face and neck.

Transposition Flaps

A transposition flap is so called because it is transposed over intervening normal tissue to reach the defect. After a time the pedicle is divided at an appropriate point and part of the flap may be returned to the place it came from. There is usually a residual secondary defect at the donor site, however, and this has to be closed, usually by a free graft.

There is no hard and fast distinction between local transposition flaps and direct flaps from a distance (p. 267)—it all depends on what one chooses to regard as local. A forehead flap used in the operation of rhinoplasty (*see* Fig. 70), for example, might be included in either category.

Z-plasty

In the operation of Z-plasty (Fig. 74) two triangular flaps are used and each fills the defect created by the formation of the other. This procedure, which is now a standard operation for the relief of certain forms of scar contracture, was devised by Denonvilliers (1857) and subsequently developed by Staige Davis (Davis 1931; Davis and Kitlowski 1939). More recently it has been used by Ferris Smith (1951) for a different purpose, namely to interchange a pathological lesion and normal skin as a preliminary to excising the lesion in multiple stages.

Relief of Scar Contracture by Z-plasty

A scar across a joint in a direction perpendicular to the axis of rotation, as Min Chyang Ju (1951) has pointed out, is subjected to great stress during muscular activity. This stress causes microscopic haemorrhages, fibrosis and eventually the

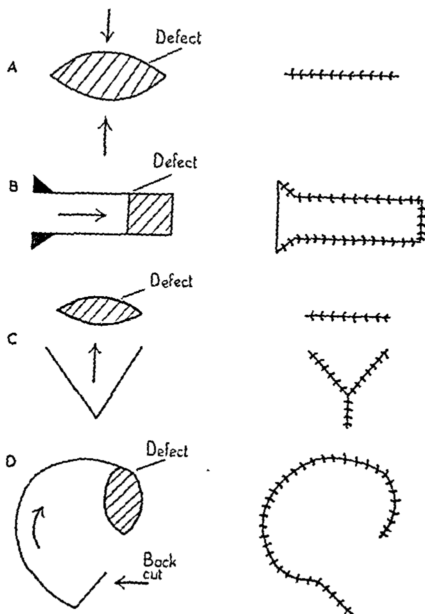


Fig. 73. Local flaps. A Sliding flaps. B Simple advancement flap. C V-Y advancement. D Rotation flap with Gillies' back cut.

formation of a dense, contracted collagenous band.

If such a band is merely excised another will form. The contracture can be permanently relieved, however, by making a Z (or reversed Z) incision with its central limb in the direction of the band, raising the two triangular flaps thus outlined, and interchanging them after excising all underlying contracted fibrous tissue, because by this manoeuvre the original scar is replaced by scars which are subject to much less stress.

Geometry of Z-plasty

The geometrical theory of Z-plasty was first studied by Limberg (1929, 1935), and his work has been made readily accessible to English-speaking surgeons by Davis and Kitlowski (1939). The theory assumes that after transposition there are no raw areas exposed, nor do the flaps overlap. It also assumes that the flaps themselves are rigid, that is they retain their original size and shape after transposition, whereas the surrounding skin is elastic. It follows that the

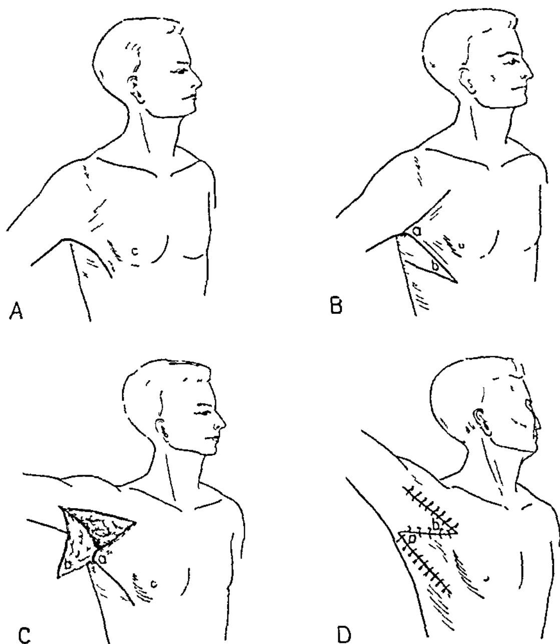


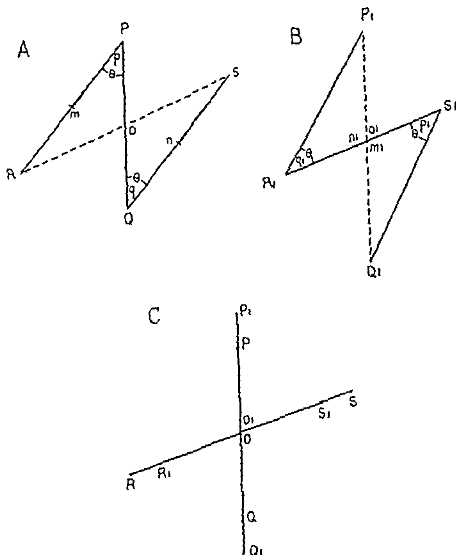
Fig. 71. Treatment of scar contracture of the axilla by Z-plasty. The small letters are to identify corresponding points in the successive drawings A, B, C, D.

central part of the Z and the two side limbs must all be equal in length. The angles between the side limbs and the central part of the Z may be equal or unequal.

Limberg's geometrical constructions are cumbersome, and his treatment of the asymmetrical Z is in one respect misleading. We shall therefore modify the argument, though we shall reach similar conclusions.

Z with Equal Angles. In the simplest case the side arms of the Z make equal acute angles θ with the central portion. We shall denote the length of each limb by x.

The appearance before transposition is shown in Figure 75A. PQ is the middle part of the Z, PR and QS are the side arms, and O is the point at which the line joining R and S meets PQ. The letters p and q denote the apices of the triangular flaps, so that in this figure p coincides with P, and q with Q. m and n are the midpoints of PR and QS respectively.



F[illegible] 75. Geometry of Z plasty with equal angles. For explanation see text.

The position after transplantation is shown in Figure 75B. Here P', Q', R', S', p', q', m', n' denote the new positions of the points P, Q, R, S, p, q, m, n. The triangles RPO, SQO, QP'm, PQn, Q'S'm', P'R'n' are clearly all congruent, from which it follows that n' and m' coincide with O', the midpoint of R'S', and that P'O'Q' is a straight line. Furthermore the angles P'O'S' and POS are equal, so that the two figures may be superimposed in such a way that P', P, Q, Q' fall on one straight line, and R, R', S', S on another (Fig. 75C).

If we denote the distance P'Q' by d, and the amount of relaxation, that is the increase in length in the direction of the scar PQ, by r we have:

$$d = P'Q' = R'S' = \sqrt{RP'^2 + PS'^2 - 2RP.PS \cos RPS}$$

$$= x\sqrt{1 + 8 \sin^2 \frac{\theta}{2}}$$

$$r = d - x$$

If, for example, $\theta = 60°$ we find:

$d \simeq 1.7x$, $r \simeq 0.7x$.

If $\theta = 76°$ then $d \simeq 2x$, $r \simeq x$.

Z with Unequal Angles. *In the general case the side arms of the Z make unequal angle θ and φ with the central portion.*

The appearance before transposition is shown in Figure 76A. Again PQ is the mid-

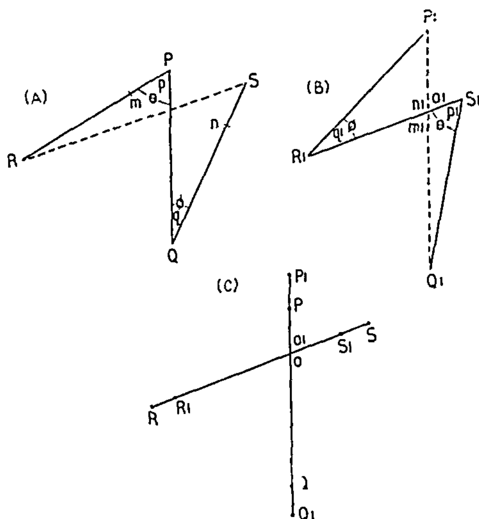


Fig. 76. Geometry of Z-plasty with unequal angles. For explanation see text

dle of the Z, PR and QS are the side arms, O is the point at which the line joining R and S meets PQ, and p and q are the apices of the flaps. m is a point on PR such that Pm = PO and n is a point on QS such that Qn = QO.

The position after transposition is shown in Figure 76B. Since Sn = pm, the points n' and m' coincide. Secondly, since angle P'n'R' = angle PnQ = angle SOQ = angle POR = angle PmQ = angle S'm'Q', the points n' and m' coincide with O', the point at which the straight line joining P' and Q' meets R'S'. Thirdly, P'Q' = P'n' + m'Q' = Pn + mQ = OS + RO = RS, and R'S' = Rm + nS = x.

As before it is easy to see that angle P'O'S' = angle POS, so that the figures may be superimposed in such a way that P', P, Q, Q' fall on one straight line and R, R', S', S on another.

If, as before, d denotes the distance P'Q' and r the amount of relaxation in the direction of the original scar, we have,

$$d = P'Q' = RS = \sqrt{RP^2 + PS^2 - 2RP \cdot PS \cos RPS}$$

$$d = x\sqrt{1 + 4\sin^2\frac{\theta}{2} + 4\sin\frac{\theta}{2}\sin\left(\phi - \frac{\theta}{2}\right)}$$

$$r = d - x.$$

If, for example, $\theta = 60°$ and $\phi = 30°$, we find $d \simeq 1.4x$, $r \simeq 0.4x$.

It is important to note that the distance h moved by R in taking up its new position R' is less than, equal to, or greater than the distance k moved by S in taking up its new

[illegible] greater than [illegible]

We [illegible]

[illegible] that RO + OP is less [illegible] greater than SO + OQ [illegible] greater than equal to [illegible] the result follows. The [illegible] is tedious except when [illegible] be given here.

Practical Considerations. In practice the [illegible] Z should not be less than 30 [illegible] greater than about 90. The Z with [illegible] the most generally useful. [illegible] retain their original size as [illegible] contract so that so [illegible] angles increase. The amount [illegible] greater than the value [illegible] formula. Empirically [illegible] Davis and Kitlowski (1939) [illegible] for a symmetrical 60 Z [illegible] equal to the theoretical [illegible] 76 [illegible] that is [illegible] R moves less than [illegible] Z or reversed Z [illegible] should be used if [illegible] is not the same [illegible] contracted band and [illegible] on the side of the [illegible]

Bilobed Flaps

[illegible] transposition [illegible] the tip of the [illegible] frontal lobe (b) [illegible] through 90 into [illegible] which was [illegible] defect. The ter[illegible] of the nose was [illegible] tissue from each side. [illegible] procedure is an alterna[illegible] he had tried previously [illegible] single flap from the [illegible] through 180 and which [illegible] unsatisfactory because twisting [illegible] extent endangered the

Fig. 77. Esser's bilobed flap. Lobe b is rotated downward through 90° to repair the tip of the nose while lobe c is rotated through 90° into the secondary defect. (Reproduced from *Deutsche Zeitschrift für Chirurgie* by courtesy of the publishers.)

viability of the flap and left considerable residual deformity.

More recently Zimany (1952) has used bilobed flaps for a different reason, namely to take advantage of the fact that, while tissue laxity is maximal in one particular direction, there is often some laxity also in the direction at right angles to this. In this event it is possible to use a bilobed flap (Figs. 78, 79) consisting of a large lobe (a) which is swung into the primary defect and a narrower lobe (b) which is swung into the secondary defect resulting from the transposition of a. If the flap is properly designed there will be sufficient slack in the direction perpendicular to the original long axis of a to enable the secondary defect to be closed by sliding in tissue from the margins to meet b and sufficient slack in a direction perpendicular to the original long axis of b to enable the tertiary defect resulting from the transposition of b to be closed by sliding alone. In the illustrations the long axes of the two lobes are at right angles but as Zimany points out the angle between these measured in the direction of rotation may have any value from 45° to more than 180°.

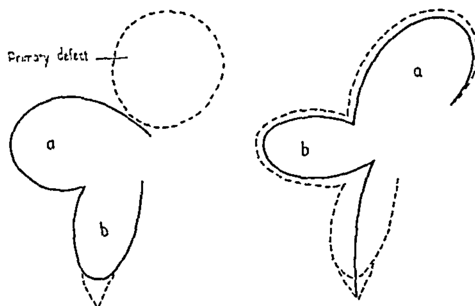


Fig. 78. Zimany's bilobed flap. The large lobe a is swung into the primary defect and the narrower lobe b is swung into the defect resulting from the transposition of a. (Reproduced from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Dr. Alexander Zimany.)

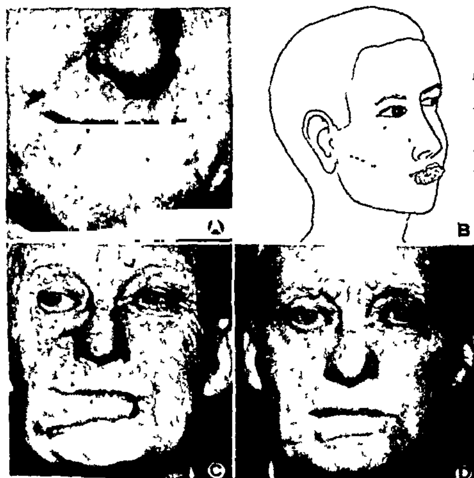


Fig. 79. Case illustrating the use of Zimany's bilobed flap. A. The lesion—an extensive carcinoma of the lower lip. B. Design of the bilobed flap for reconstruction after excision of the lesion. C. The reconstruction. D. The end result. (Reproduced from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Dr. Alexander Zimany.)

Lined and Buried Flaps

Repair of Defects Involving Skin and Mucous Membrane

A lined flap is one which has epithelium on its deep surface as well as on its superficial surface. Lined flaps may be used to repair defects involving both skin and mucous membrane, such as occur with full-thickness wounds of the cheeks or nose. The lining may consist of a free graft applied to the deep surface of the flap, or may be formed by folding part of the flap back on itself or by turning in additional local flaps at the margins of the defect.

Cutaneous Oesophagoplasty

Under this heading we shall consider firstly the use of local skin flaps in reconstruction of the cervical oesophagus and pharynx, and secondly the construction of antethoracic and intrathoracic skin tubes and their use as bridges between the cervical oesophagus and the stomach. Many of the procedures to be described are now obsolete, and the remainder seem likely to become so before long, but they are of considerable historical importance and interest.

Reconstruction of the Cervical Oesophagus. Mikulicz (1886) resected the cervical oesophagus for carcinoma and four months later closed the fistula resulting from this operation and reconstructed the oesophagus with two skin flaps, one from each side of the neck, which he united to form a tube. The skin lateral to the flaps was drawn together over the tube with silver wire sutures. The wound healed well and ten days after the operation the patient was able to swallow solid food, but death occurred from recurrence of the tumour sixteen months later Von Hacker (1891), after experimenting in dogs, attempted a similar procedure but the patient died before it was complete; later, however, he was able to report a successful case (von Hacker, 1908).

Lane (1911) introduced a new technique (Fig. 80) in which he used a single flap with its base to the left of the neck. After resecting the tumour with a margin of healthy oesophagus he rolled up the flap to form a tube in the long axis of the oesophagus and joined it to the pharynx above and to the oesophagus below. At a second stage he divided the base of the flap and closed the lateral aperture remaining in the tube. The patient did well for a time but died seven months later from recurrence of the growth.

Lane's procedure has continued to be used but since 1912, when it was re-described by Wookey, it has usually been referred to as Wookey's operation (*see also* Wookey, 1948; E. Lewis, 1952; Owen, 1952).

When reconstruction is postponed and the patient presents with a pharyngostomy and an oesophagostomy created at the time of the resection, a simpler procedure similar to that used in antethoracic oesophagoplasty (*v. infra*) can be used. One such case was reported by J. R. Lewis in 1950.

Antethoracic Skin Tubes. In 1894 H Bircher attempted to short-circuit a carcinoma of the oesophagus by means of a buried skin-lined tube. At the initial operation he made two vertical incisions about three inches apart on the front of the chest just to the left of the midline, undercut the edges of the strip of skin thus isolated without disturbing the attachment of its central part and united them to form a tube (Fig. 81). He then mobilized the skin on each side of the defect and sutured the margins together so as to bury the tube. At a second operation Bircher united the lower end of the skin tube to the stomach via a gastrostomy. He planned to unite the upper end of the tube to the oesophagus in the neck, but the patient died before this was done. Bircher made a second attempt in another case but once again the patient died before the procedure was complete. These cases were not reported at the time but were published in 1907 by E. Bircher.

According to Saint (1929) this form of oesophagoplasty was completed for the first time by Payr in 1917. Since then it has been used with varying degrees of success to short-circuit simple or carcinomatous strictures of the oesophagus and to restore continuity after the now obsolete Torek and Grey Turner types of oesophagectomy (Rovsing, 1925, 1926; Braizew, 1929; Grey Turner, 1933; King, 1936; Wookey, 1940; Stevenson,

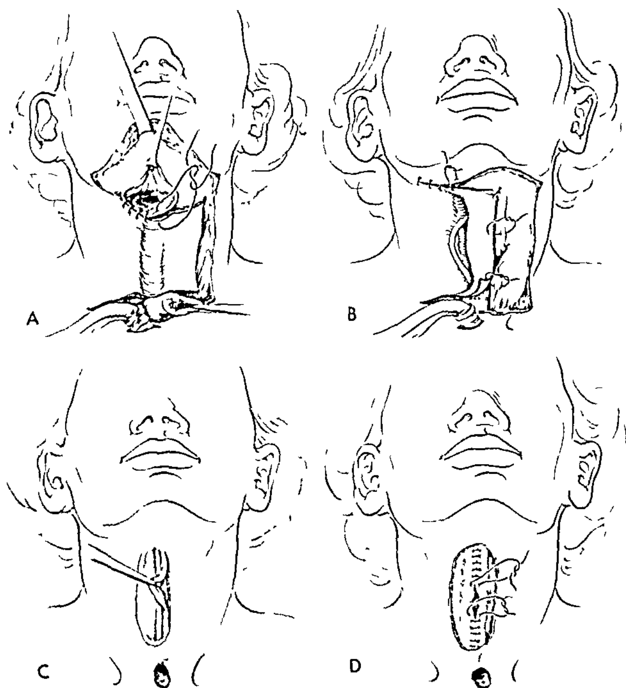


Fig. 80. Wookey's (1942) elaboration of Lane's method of reconstructing the pharynx and cervical oesophagus by means of a skin flap. A. Suturing the flap to the wall of the pharynx. B. The first stage of the operation has been nearly completed. The flap has been sutured to the pharynx above and to the oesophagus below, so that it forms a tube with a longitudinal opening towards the right. The remainder of the flap has been folded back to close the raw surface as far as possible, after which the rest of the defect will be covered with split-skin grafts. C, D. Closing the lateral fissure at a subsequent operation. (Reproduced from *Surgery, Gynecology and Obstetrics* by courtesy of the editor and Dr. Harold Wookey.)

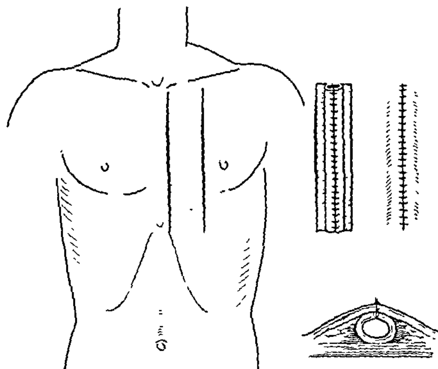


Fig. 81 Bircher's method of constructing an antethoracic skin tube to replace the thoracic part of the oesophagus.

1947 Shaw, 1950, and others). It has also been used in the treatment of congenital atresia of the oesophagus when direct anastomosis after resection was not possible (Ladd 1944, Ladd and Swenson, 1947; Ivy, Hawthorne and Ritter, 1948)

Bircher's technique has been modified in various ways Esser (1917c) formed a skin tube by burying split skin grafts, wrapped raw surface outwards around a cylindrical mould, in a subcutaneous tunnel Galpern (1925) tried without much success to use omentum to provide a serous covering for the lower end of a tube constructed by Bircher's method Rovsing (1925, 1926), apparently unaware of Bircher's work, described a very similar procedure, the main difference being that he brought the aboral stump of the cervical oesophagus to the surface as a fistula, instead of closing it, in order to avoid the danger of leakage into the mediastinum

To overcome the difficulty of burying the substitute oesophagus with local skin Davis and Stafford (1942), Ladd (1944), Ivy, Hawthorne and Ritter (1948) and others used a tubed-pedicle flap from the lateral thoraco-abdominal region for this purpose. Hanrahan (1947), in a patient in whom a jejunal loop brought up to a cervical oesophagostomy had partly broken down, found that the skin between the oesophageal and jejunal openings was too scarred to be used to form a tube. He therefore raised a long tubed pedicle graft and used part of this to replace the scarred skin. Next he used the transplanted skin to form a substitute oesophagus, and finally he buried this with the remainder of the tubed pedicle graft.

Intrathoracic Skin Tubes. Attempts have been made to repair defects in the thoracic oesophagus with skin flaps applied as patches, and to replace a segment of the oesophagus with a skin tube transplanted to the mediastinum and joined to the oesophagus above and below by end-to-end anastomosis (Bricker, Burford and Eiseman,

1949). These procedures are however difficult and hazardous, and in patients who survive there is a high incidence of post-operative stricture (Rubenstein, 1956).

Whatever technique is used there is a great tendency to fistula formation, especially at the junction of skin tube and stomach. For this and other reasons cutaneous oesophagoplasty has been combined with and more recently largely superseded by methods involving pedicle transplantation of stomach or bowel (Chapter 27).

The Use of Local Flaps to Augment the Blood Supply of a Part

Sometimes when the defect to be repaired is in an area where the circulation is poor the decision to use a local flap rather than a flap from a distance is based on the necessity for retaining an intact pedicle as a source of permanent blood supply. In this event, as Brown, Fryer and McDowell (1951 a, b) have suggested, the flap may be aptly described as a "permanent pedicle blood-carrying flap".

The use of local flaps in this way suggests the possibility of using skin flaps to augment the blood supply of viscera, and in particular to augment the blood supply of the heart in coronary arterial disease (p. 113). This does not appear to have been tried as yet in man, but Moran and his colleagues (von Wedel, Stone, Neumann, Lord, Hinton and Moran, 1952; Moran, 1952b; Neumann, Moran, von Wedel, Lord, Stone, Wade and Hinton, 1953) have investigated the problem in dogs, using a thoracoabdominal pedicle, tubed in its proximal part and attached at its distal end to the myocardium. One month after operation they were able to demonstrate numerous anastomoses between the vessels of the flap and the coronary vessels by radiographic studies following injection of an opaque medium; they showed too that in animals protected by flaps the descending branch of the left coronary artery could be ligated without mortality.

More recently Conway and Griffith (1957) have investigated in dogs the possibility of using skin flaps to provide an alternative blood supply to the kidney. In these experiments one kidney was decapsulated and attached over its whole surface to the opened-out end of a previously prepared tubed pedicle graft. The renal vessels were subsequently divided and two weeks after this the opposite kidney was removed. The dog remained in good health and voided urine regularly. An aortogram did not show any vessels passing from the aorta to the kidney, but in a radiograph taken four minutes after the injection the renal pelvis was filled with dye. The animal was killed and it was shown by injection studies and histological examination that the kidney had acquired an extensive blood supply via the flap.

FLAPS FROM A DISTANCE

Repair by flaps from a distance is usually a more complicated matter than repair by local flaps; it has the advantage however of allowing some freedom of choice of the donor area, and may be the only feasible procedure when the defect is large and the surrounding tissue is unhealthy.

Flaps from a distance take many forms; they may however be grouped under four main headings:

1. Simple flaps.
2. Tubed pedicle flaps.
3. Island flaps.
4. Compound flaps.



Fig 82 Direct flap from the abdomen used to repair a defect on the anterior aspect of the elbow A Raising the flap B. The flap in place at the end of the first stage of the operation C Completing the repair after dividing the pedicle and trimming the flap.

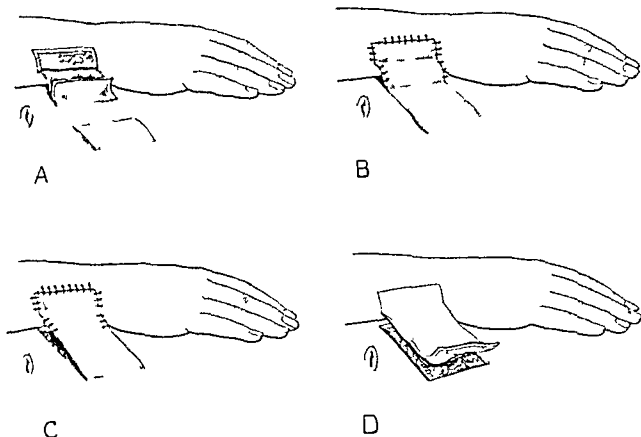


Fig 85 Mustardé's technique for rapid transfer of an abdominal flap A B Flap undermined but left attached to the donor area at one end and by a bridge on each side Free end of the flap attached to the wrist by the trap-door method C Bridges divided but one end of the flap still attached to donor area D Transfer completed (Redrawn from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Mr J C Mustardé)

Simple Flaps

A simple flap may be transferred to the recipient site directly (Fig 82) or through an intermediate attachment Direct transfer is more common because when transfer via an intermediate site is necessary it is often best to use a tubed flap A simple flap may be transferred via an intermediate attachment without danger of infection however, if the exposed raw surface is temporarily covered with a split skin graft as in Mustardé's (1953) procedure (*see infra*)

The secondary defect at the donor site can often be closed by local sliding flaps and failing this a free split skin graft is generally used Another procedure devised by Moore and Faulkner (1951) is to return the superficial part of the flap to the donor site leaving the deep part at the site of the primary defect and cover this deep part with a split skin graft

The viability of the distal end of a flap which is raised in one operation depends on the site of the donor area and the ratio of length to breadth of the flap A good example of a favourable site is the temporal region from which it is possible to fashion a flap containing the temporal artery and vein (*see* Conway Stark and Kavanaugh 1952) The permissible maximum ratio of length to breadth varies but may be less than one and is rarely greater than two

When a long narrow flap is required or the blood supply to the donor area is poor, a flap raised in one operation will necrose it may still be possible however to raise a flap successfully by delay (p 252) Climo (1951) has suggested that when a flap is

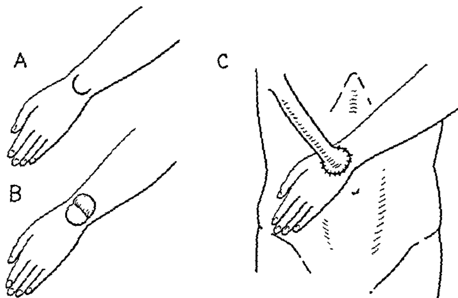


Fig. 84 *The trap door technique for attaching a tubed pedicle graft temporarily to the wrist. A curved incision is made on the dorsum of the wrist (A) and the small proximally based flap which is thus outlined is turned upwards (B). The distal end of a previously constructed abdominal tubed pedicle graft is freed and sutured to the margins of the incision and the wrist flap (C).*

being raised it is possible to judge accurately whether it can safely be transferred without delay by observing the colour of the blood escaping from severed dermal vessels. Constant *red* dermal bleeding from the edges of the flap is good evidence of an adequate circulation. On the other hand *blue* dermal bleeding, according to Climo, even though localized to a small segment of the periphery, is evidence of a deficient venous return, and is an indication to stop raising the flap and return it to its bed.

A valuable procedure for transferring an abdominal flap via the wrist, which uses the principle of delay but avoids unnecessary waste of time, has been described by Mustardé (1953). Incisions are made as shown in Figure 83, leaving a bridge on each side; the flap is then undermined but remains attached at one end and by the bridge on each side. A split skin graft is draped, raw surface outwards, round a sheet of thin soft rubber of appropriate size, and the whole is inserted beneath the flap so as to provide epithelial cover for both the deep surface of the flap and the bed from which it was raised. Finally the distal end of the flap is attached to the wrist by the usual trap-door technique (Fig. 84). In this way the process of transfer is started before the whole flap is ready to be raised.

The value of delaying a flap is beyond dispute, but its rationale is less certain. It is commonly stated that new blood vessels develop in the intervals between the successive operations by which the flap is fashioned; Hynes (1950) has denied this, however, and attributes the success of a delayed flap to dilatation of existing vessels in the flap and consequent reduction in the resistance to blood flow. This dilatation occurs because vessels, and with them perivascular sympathetic nerve fibres responsible for maintaining vessel tone, have been divided during the process of raising the flap. These nerve fibres also supply sweat glands, and evidence of their division is provided by Hynes' demonstration that the skin of a recently constructed flap is incapable of sweating.

Hynes' explanation is open to the criti-

claim that in peripheral vascular surgery, if the patient's vessels are capable of dilating at all, lumbar sympathectomy *produces an* almost immediate increase in the blood flow to the skin. Why, then, should the peri-arterial sympathectomy which, *ex hypothesi*, occurs when a flap is raised be more effective if performed in stages? The matter could be subjected to a crucial test because, if Hynes is correct, any lower limb flap which could be successfully raised by delay could be raised equally successfully in one operation after a preliminary lumbar sympathectomy. This test has not been rigorously applied, but Kallio (1919) has used *lumbar sympathectomy prior to flap* grafting and other plastic operations on the lower limbs in a few patients, and his findings lend some support to Hynes' case.

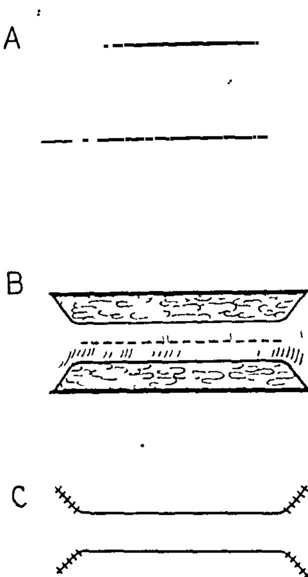


Fig. 85. Usual method of constructing a tubed pedicle graft. A. Two parallel incisions are made through skin and subcutaneous fat, and the flap thus defined is raised from the underlying tissue but left attached by a bridge at each end. The skin and subcutaneous tissue on each side of the flap is extensively undermined. B. The margins of the flap are sutured together so that a tube is formed with the skin surface outwards. C. If possible the defect is closed by sliding in local flaps; failing this a split skin graft is used.

Tubed Pedicle Flaps

By tubing a pedicle flap complicated manipulations are facilitated and the risk of infection is diminished. The usual method of construction is illustrated in Figure 85; it has the disadvantage that the tube overlies the donor area, making it difficult to close the latter, and to avoid this Gabarro (1943) *devised the method shown in Figure 86*. Another modification, suggested by Maltz (1939), is to sew up the tube with the raw surface outwards in order to overcome the tendency to curling which occurs after transfer of the usual type of flap; it seems quite unjustifiable, however, to court infection by exposing subcutaneous tissue in this way.

Whatever method of construction is employed the tube is normally left attached at both ends for a time; subsequently one end is divided and swung into the defect or to an intermediate attachment.

The tubed pedicle flap has many applications in plastic surgery, especially in the treatment of defects involving the neck and face. Besides being used extensively for replacing lost skin and subcutaneous tissue (Fig. 87) it provides an excellent means of *repairing full thickness defects of the lips or* cheeks caused by excision of tumours or radiation necrosis (Ivy 1949; Catlin 1950). It has also been utilized by Kostrubala (1950) for repairing the palate (Fig. 88).

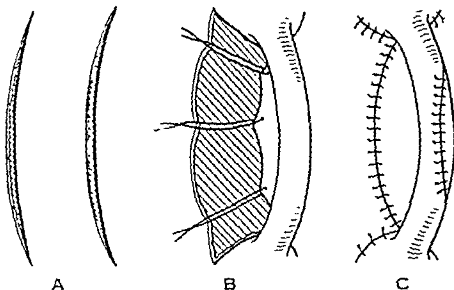


Fig. 86. Gabarro's method of constructing a tubed pedicle graft. A. Two parallel curved incisions are made instead of the usual straight ones. B. The graft is tubed and retracted to one side. The stitches used to close the defect are orientated radially. C. Further sutures are placed as shown to complete the procedure. (Redrawn from *Surgery* by courtesy of the publishers and Dr. P. Gabarro.)

Island Flaps

An island flap is a flap in which the pedicle contains blood vessels but no skin, and only a minimal amount of subcutaneous tissue (Fig. 89). It is fashioned by dissecting out a disc of skin and a sufficient length of vascular pedicle, and is moved to its new site via a subcutaneous tunnel or, if it is too large to permit this manoeuvre, by extending the incision as far as the defect. In either case the pedicle is buried and remains permanently as a source of blood supply.

The island flap was described first by Gersuny (1887), and later by Monks (1898) and Esser (1917b). It is used occasionally today for repairing small defects on the face. Ferris Smith (1951), for example, has used an island flap supplied by the frontal artery for repairing defects on the side of the nose.

Compound Flaps

A compound flap is one which contains bone, cartilage, muscle or mucous membrane in addition to skin and subcutaneous tissue. It will suffice to mention two examples.

The Stein-Estlander-Abbé flap is a pedicle flap of skin, muscle and mucous membrane transferred from one lip to the other.

Stein (1848) used a flap of this kind from the upper lip to repair a defect in the lower lip due to cancer. Estlander (1877) reversed the procedure and used a flap from the lower lip to repair a defect caused by gangrene in the upper lip. Abbé (1898) used a V shaped flap from the lower lip for the secondary repair of hare lip in patients where the primary operation had resulted in a taut flattened upper lip (Fig. 90). The value of Abbé's operation was not fully appreciated at the time but is now widely recognized.

Cole's flap consists of skin, platysma muscle and clavicular bone. It was designed (Cole, 1918) for repairing defects involving the mandible and the overlying skin, but has become obsolete because defects of the mandible are nowadays usually repaired by free grafts of iliac bone (Chapter 18).

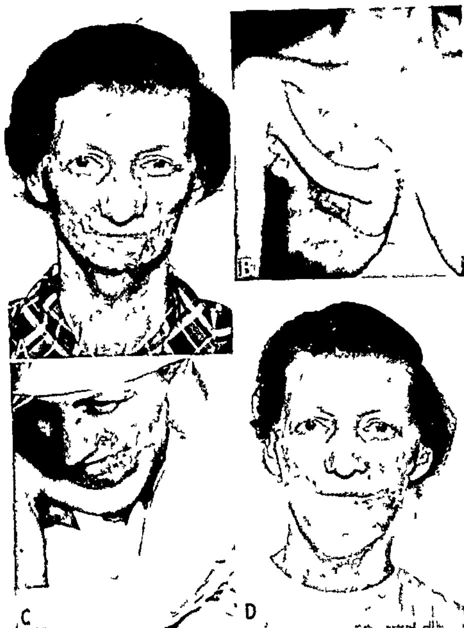


Fig. 87 Acromio-thoracic tubed pedicle graft used to repair the defect resulting from excision of a squamous-celled carcinoma on the chin which developed in a patient with extensive lupus vulgaris. A Before operation. B The graft raised. C Lower end of graft transferred to the chin. D Final appearance. (Mr A B Wallace's case.)

Evaluation of the Circulation in Flaps

Accurate evaluation of the circulation in a flap which is to be migrated is of great practical importance. If the flap is moved too soon it may necrose; on the other hand by waiting longer than is strictly necessary the patient's stay in hospital is needlessly prolonged. Experimental investigations have thrown much light on this problem and tests have been devised which, while they are no substitute for clinical judgement, are of considerable value in reconstructive surgery.

Experimental Investigations

Stage Davis and his colleagues (German, Finesilver and Davis 1933) appear to have been the first to make a systematic study of the development of new vessels in tubed



Fig 88 Use of a tubed pedicle graft to repair an extensive defect of the palate caused by a gunshot wound. The anterior part of the alveolar ridge had also been destroyed and the graft was led in through this defect after detaching the nose from the left side of the face. (Dr. Joseph G. Kostrubala's case)

pedicle flaps in experimental animals (dogs). By means of dye injection radiography after injection of radio-opaque substances, and ordinary histological methods, they showed that new vessels were established within seven days after fashioning the flap and changed little in number or size between the seventh and fourteenth day.

Conway, Stark and Docktor (1949), using radiography after intravascular injection of 26 per cent colloidal thorium dioxide combined with histological methods, confirmed and extended these observations, in addition they investigated the earliest date at which a flap could be successfully migrated. Their findings may be summarized as follows:

1. Between the 10th and 19th days the longitudinal vessels in the tube did not increase in number, though they increased in length as indicated by increased tortuosity.

2. Despite this the flap could be migrated after 3 weeks, and sometimes as early as the 9th post-operative day.

3 After 4-5 weeks there was a marked increase in the number of longitudinal vessels and branches.

Braithwaite (1950) used micro-radiography after injection of Pyelosil or 20 per cent colloidal silver iodide (*see* Barclay, 1947), to study the vascular channels in human skin. He examined normal skin and also skin from tubed pedicles, and showed that the dermal and subdermal vascular plexuses

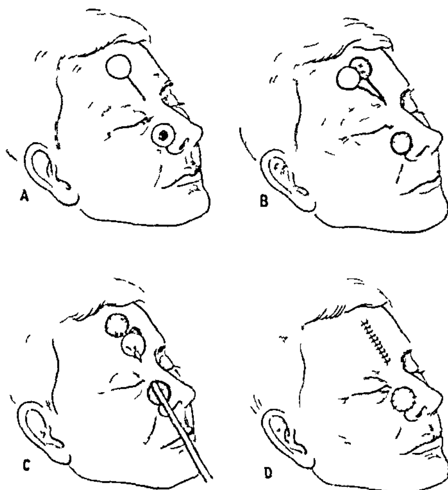


Fig. 63. Island flap. A. Basal-cell carcinoma on nose showing the area to be excised and the incision for raising the flap. B. The lesion has been excised. The flap has been raised but retains its blood supply from the supratrochlear artery. *C. The flap is being drawn down to the defect through a subcutaneous* tunnel. D. Completion of the operation.

played a dominant part in maintaining the circulation in a pedicle graft.

Tests Designed for Clinical Use

The tests available for clinical use have been reviewed by Conway (1952). They are for the most part modifications of tests used for assessing the circulation in the limbs in patients with peripheral vascular disease. The following is a convenient classification:

1. *Tests designed primarily for determining when a tubed pedicle flap may be safely migrated.*
   (1) Tests depending on changes in temperature following application or release of a tourniquet.
   (2) Measurement of the rate of return of systolic blood pressure after release of tourniquet.
   (3) Photo-electric determinations of changes in hue after application of a tourniquet.
   (4) Visualization of the circulation in the flap by observation under ultraviolet illumination after intravenous injection of fluorescein.
   (5) Wheal tests.
      (a) Saline wheal.
      (b) Histamine wheal.
   (6) Studies of the rate of absorption of substances injected into the flap.

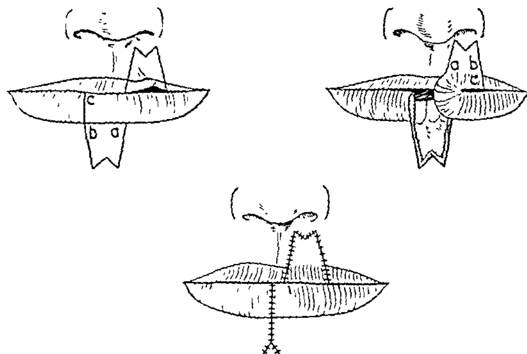


Fig 90 The Stein Estlander Abbe flap The flap is obtained from the lower lip and used to repair a defect in the upper lip, resulting in the case illustrated from excision of a scar. The letters are for identification of corresponding points in successive drawings.

(a) Hynes' atropine test
(b) Conway's radioactive sodium clearance test.

2 *Olander's test for determining the source of blood supply in delayed flaps*

Tests Depending on Changes in Temperature. Douglas (1942) recorded with a thermocouple the rate of return of temperature in a tubed flap after release of tourniquets previously applied to both ends of the tube Conway, Stark and Docktor (1949) used a more elaborate test, observing the skin temperature at a point near one end of a tubed pedicle before and after application of a tourniquet at the same end. The second test in particular appeared promising but Conway (1952) has now concluded that it is not reliable.

Tests Based on the Rate of Return of Systolic Blood Pressure and on Changes in Hue. Douglas and Bucholz (1943) observed the rate of return of systolic blood pressure after release of a tourniquet applied to a pedicle, and Douglas and Millikan (1947) used a photo electric oximeter to record changes in hue. Neither test is entirely reliable

The Fluorescein Test. Lange and Boyd (1942) assessed the circulation in superficial tissues by injecting fluorescein intravenously and then observing the extent to which the area fluoresced under ultraviolet illumination. Subsequently Dingwall and Lord (1943) applied this method to tubed pedicle flaps, and concluded that a flap could be migrated if it showed uniform fluorescence while a tourniquet was in place on the end to be divided.

Lange (1944), using the same test experimentally in rabbits, found that there was evidence of capillary connexion between the recipient site and a pedicle flap within 144 hours, and full vascularization in about 8 days. He injected 10 ml. of an aqueous solution of 50 per cent fluorescein and 5 per cent sodium bicarbonate, and illuminated

the skin with a mercury vapour lamp provided with a filter transmitting only light with a wave length of about 3,600 Angström units.

The Saline Wheal Test. Conway, Stark and Docktor (1949) investigated the circulation in tubed pedicles by a modification of the saline wheal test designed by Stern and Cohen (1926) for studying the circulation in the legs of patients with vascular disease. To perform the test a tourniquet is applied to the end of the tube which is to be divided and a wheal 1 cm. diameter is formed by injecting isotonic saline intradermally at a point on the tube close to the tourniquet. A similar wheal is made in a control area and the rate of disappearance of the two wheals is compared. The objections to the test are that it takes about an hour to perform and the end point is not sufficiently definite.

The Histamine Wheal Test. The histamine wheal test, used by Starr (1928) and de Takats (1929) in the study of peripheral vascular disease, has been adapted for the evaluation of the circulation in flaps by Conway, Stark and Joslin (1951b). With a tubed flap the end to be divided is constricted by a tourniquet and several small areas near the same end of the flap are scarified with a needle; a drop of a 1 in 1000 solution of histamine acid phosphate is then applied to each area and the time which elapses before wheals develop is recorded. According to the originators of the test the flap may safely be migrated if this time is less than 8 minutes; it is wise however to perform a similar test in an area of normal skin as a control. The procedure is simple and reliable, and could with advantage be used more often than it is.

It is of interest that a flare reaction, such as occurs in normal skin, does not occur if the histamine test is performed near the centre of a tubed pedicle. This observation supports Hynes' (1950) contention that the sympathetic nerve supply to the region in question is destroyed.

Hynes' Atropine Test. In this test (Hynes, 1948) the circulation at the end of the flap which is to be divided is occluded with a tourniquet, and one-twenty-fifth to one-fiftieth of a grain of atropine is injected subcutaneously into the flap. If the circulation at the other end is adequate the usual effects of the drug are observed, such as tachycardia, dryness of the mouth, and paralysis of visual accommodation. In practice reliance is placed on the development of tachycardia, and a rise in pulse rate to 140 per minute within 40 minutes is taken to indicate that the flap may safely be migrated. The test takes rather a long time to perform but appears to be reliable.

Conway's Radioactive Sodium Clearance Test. This test, developed by Conway, Roswit, Stark and Yalow (1951), is based on a procedure used by Kety (1949) for the investigation of peripheral arterial disease. 0.05 ml. of isotonic saline containing 0.1 to 0.5 microcuries of radioactive sodium (Na^{24}), which emits beta particles and gamma rays, and has a half life of 14.7 hours, is injected intradermally at a point near the end of the pedicle which is to be divided; as a control a similar injection is made in the skin of the corresponding region on the opposite side of the body. A Geiger counter with a thin mica window, which detects beta particles and, less efficiently, gamma rays, is placed over the injection site and the counting rate is recorded as a function of time. In theory, complete absorption takes an infinitely long time, but normally after about half an hour the quantity remaining is negligible. Occasionally some of the solution is accidentally deposited on the skin; in this event when absorption is virtually complete the counting rate does not fall to zero but becomes constant at a value termed the background rate. The net counting rate at any given time is defined as the observed

rate at that time minus the background rate If the net rate is plotted against time on semi-logarithmic paper the resulting graph is, within the limits of experimental error, a straight line, and from it the half-period for absorption can be read off In control areas this period is on average about 60 seconds and if the same or a lower value is obtained from a pedicle with neither end occluded it is safe to proceed with division and migration If a similar test is performed while the vessels at one end of the pedicle are occluded with a tourniquet, the half-period for absorption may be 45 to 60 minutes Performance of the test in this way is a refinement which according to Conway is rarely called for in practice

If the necessary facilities are available the radioactive sodium clearance test is well worth using and will often enable a pedicle to be transplanted after 14 to 18 days when without the test most surgeons would deem it prudent to wait for 3 to 4 weeks

Olander's Test. Olander (1950) devised this test to determine the source of blood supply at any given stage during the construction of a delayed flap The patient is given a half to one grain of nicotinic acid by mouth to produce cutaneous vasodilatation and adrenaline solution (1 in 30,000) is then injected in the vicinity of the operation scars which outline the flap but not into the flap itself Local blanching results, and if the blanching extends across the scar into the flap it indicates that the blood supply of the flap in this region is derived from the adjacent tissue, and transplantation should be postponed

Attempts to Improve the Circulation in Flaps

Procedures Designed to Expedite Revascularization

Revascularization of a tubed pedicle flap may be hastened by intermittently occluding the end which is to be transplanted with a rubber tourniquet until cyanosis develops The procedure is carried out daily for a few days, and then twice or thrice daily. According to Wong (1951) the flap may safely be migrated when the tourniquet can be left on for half an hour or longer without causing any marked change in colour. A refinement of this procedure has been devised by Hynes (1950), who has applied to flaps the method of mechanically controlled intermittent vascular occlusion used in the treatment of peripheral arterial disease.

The Treatment of Threatened Necrosis

A flap which is transplanted prematurely loses its healthy pink colour and becomes either blue or white. A blue flap is often said to indicate venous insufficiency, a white flap arterial insufficiency. Hynes (1950) has pointed out, however, that the colour of a flap may be altered by changes in posture, and claims that blue and white flaps are both due to arterial insufficiency.

Impaired circulation results in tissue anoxia, and may be followed by oedema, intravascular clotting and necrosis Prompt treatment is therefore required.

Wallace and Learmonth (Learmonth, 1946) have succeeded in improving the circulation in flaps by heating the whole body and thus producing reflex dilatation.

Another simple but useful procedure (Hynes, 1950), which is applicable to flaps inset on a limb, is to raise and lower the limb alternately every fifteen or twenty minutes. When the limb is lowered blood enters the passively dilated vessels of the flap and the flap becomes blue; when the limb is raised blood flows out and the flap becomes white. Stasis is thus prevented and the risk of clotting is diminished.

Wong (1951) tried injecting heparin into the flap near its distal end, and reported good results. He chose to inject heparin directly into the flap instead of giving it systemically because he hoped to obtain a more prolonged action in this way, and be-

cause he believed that heparin administered locally is capable of dissolving clots The latter belief is erroneous, and it would seem worth investigating the effect of systemic administration of heparin and other anti coagulants on cyanotic flaps Clearly, how ever the procedure *would be of clinical* value only if the advantages to be gained outweighed *the disadvantages* entailed by the risk of haemorrhage

Other procedures which merit investiga

Cooling is suggested because it depresses the metabolism of a part and may therefore assist survival under conditions of reduced blood supply Hyaluronidase facilitates the absorption of fluids administered subcuta neously it is conceivable therefore that injection of this enzyme into a blue oedema tous flap might reduce the oedema thereby lessening the pressure on the vessels of the flap and improving the circulation Injec tion of fibrinolysin either *locally or* system ically is suggested in the hope that it might *promote lysis of clots* As these would be *small there would seem to be very little risk* of serious embolism resulting

Recovery of Sensation and Sympathetic Activity

When a flap is raised and transferred to a distant site its nerve supply is interrupted it therefore becomes anaesthetic and sym pathetic activity is abolished

Recovery of Sensation

Kredel and Evans (1933) who were the first to make a *systematic study of the re* covery of sensation in flaps found that pain touch and temperature sensibility returned independently and in that order Recovery began at the periphery and progressed distally from the base and inwards from the edges of the flap These findings have been confirmed by Davis and Kitlowski (1934) Davis (1934) and McCarroll (1938) In the three cases *studied in* detail by McCarroll pain sensibility returned after an interval *ranging from* 65 to 124 days and touch sensibility after 95 to 158 days completely *normal* sensation including normal tactile discrimination was restored after 218 to 396 days

Hutchison Tough and Wyburn (1949) measured *the threshold to touch stimulus* and plotted the pattern of the touch spots in pedicle grafts of various ages from 14 months to 18 years Their results suggest that under optimal conditions the grafted skin tends to assume the sensory pattern of the skin of the region to which it has been transferred Skin transferred from the abdo men to the face for example assumes the sensory pattern *of face skin The authors* suggest that most of the sensory end organs *in the graft degenerate* and that the regen erating nerve fibres for the most part form *new end-organs* This may help to explain their findings but as they point out sensa tion is a cortical function and the change in central connexions is probably the main factor influencing the pattern of sensation *in the graft It seems likely therefore that* when abdominal skin is transferred say to a finger it assumes the sensory pattern of finger skin mainly because it is then pro jected on to a different and much larger area of cortex than it was before

Recovery of Sympathetic Activity

Evidence of sympathetic function in the skin is provided by vasomotor pilomotor and sudomotor activity

Sudomotor activity manifested as sweat ing in response to heating can be deter mined in a roughly quantitative way by means of materials which change colour in the presence of water or by measuring the electrical resistance of the skin and pro- vides an excellent index of sympathetic function since there is good evidence that the sweat glands are activated only by *nerve impulses* (*Kuntz* 1934)

McGregor (1950) studied sweating in normal skin, and in skin transferred as a pedicle graft from the abdomen to a finger 7-16 months previously. The subject's feet and legs were immersed in hot water (116° to 118° F) A solution of 3 per cent iodine in spirit was applied to the skin to be tested, using a rubber stamp 1 cm. square. When dry the painted area was pressed against a piece of starch-impregnated paper for about 15 seconds; the paper was then removed and 5 seconds later the skin was pressed against a second piece of paper This procedure was repeated until the iodine on the skin was exhausted, then, if further prints were needed, more iodine was applied If sweating occurred the prints showed a series of blue spots, each spot indicating the orifice of a sweat gland which had functioned during part or all of the 20 seconds since the previous print was obtained The number of functioning glands in the area could therefore be determined by counting the number of spots on the print, a procedure most easily carried out under low-power magnification In studying recipients of pedicle grafts, simultaneous readings were taken from the graft, a finger of the opposite hand and an area corresponding to the donor site on the opposite side of the abdomen The findings showed that the pattern of distribution of the sweat glands in the grafts remained similar to that of abdominal skin and there was no evidence of new sweat gland formation

The number of functioning glands per unit area in the graft was often only about half that in the donor region. McGregor considered three possible reasons for this: (a) loss of sweat gland elements during transplantation of the skin, (b) failure of some of the glands to become re-innervated, and (c) failure of some re-innervated glands to resume function under the conditions of the test because the graft had been transplanted to a region in which the most effective functional stimulus was not thermal but emotional He did not investigate the third of these possibilities directly because, as he said, "the various means of inducing emotional sweating are not suitable for use with hospital patients", but indirect evidence that the grafts become emotional sweating areas was obtained from a study of the time relationships of the sweating reaction to a thermal stimulus. It was therefore concluded that although a graft retained the anatomical pattern of the donor site it acquired the physiological sweating characteristics of the recipient site.

The Transport of Lymph by Flaps

Gillies and Fraser (1935) successfully treated a patient with hereditary chronic lymphoedema of a lower limb by constructing a bridge out of a large flap from the arm and forearm. The distal part of the flap, which had previously been near the wrist, was attached to the thigh, and the proximal part to the trunk above the level of the umbilicus The valves of the lymphatics of the flap were thus correctly orientated to permit lymph to pass from the limb to the upper part of the trunk, whence it drained to the axilla. A similar but less extensive operation has been used by Mowlem (1948). Attempts have also been made to treat chronic lymphoedema of the arm following radical mastectomy by flap grafting, and some good results have been reported.

PARABIOTIC SKIN FLAPS

Parabiotic skin grafts have been discussed in Chapter 3. From a biological point of view they are of great interest, but in our present state of knowledge they should *never* be used clinically because they are not only useless but dangerous. If a parabiotic skin graft is made one or other partner becomes acutely ill a few days after operation and recovers only when the pedicle is divided. Following this division the graft soon becomes necrotic.

PEDICLE GRAFTS OF MUCOUS MEMBRANE

Kiehn (1951) has used pedicle grafts of buccal mucosa to repair parotid fistulae in two patients and in reconstruction of the upper lip following excision of a squamous cell carcinoma. He has suggested that such grafts should be used more extensively in reconstructive surgery.

CHAPTER 15

Transplantation of Adipose Tissue, Connective Tissue and Tendon

In this chapter we shall consider the transplantation of adipose tissue, fascia and tendon; and, in addition, the heterotopic transplantation of dermis and whole skin.

TRANSPLANTATION OF ADIPOSE TISSUE

History

Neuber (1893) and Silex (1896) used small free transplants of adipose tissue successfully in treating depressed scars in the vicinity of the eye. Massive transplants in Neuber's hands were unsuccessful, but in 1895 Czerny reported successful autotransplantation of a large lipoma to replace a breast.

Free transplants of adipose tissue were used to fill cavities in bone by Makkas ([illegible]), Chaput (1901, 1913), Lawrowa ([illegible]), Binnie (1911) and others but this procedure was soon abandoned. They were used by Hoffa (1906), Chaput (1912) and Röpke (1913) in arthroplasty.

Verderame (1909) was the first to describe the shrinkage of adipose tissue transplants and to advise that allowance should be made for this.

Boljarski (1910), followed by Stuckey (1912), Hilse (1911) and Binnie (1914), used free transplants of adipose tissue to control haemorrhage from solid viscera, but it was soon shown by Risley (1917) that muscle transplants were much more effective. Tuffier (1913a) used a large transplant of adipose tissue to collapse the lung in a patient with bronchiectasis.

Rehn (1912, 1913a), working in Lexer's department, studied the behaviour of autotransplants and homotransplants of adipose tissue in rabbits and dogs. After autotransplantation part of the fat persisted unchanged; in the remainder cystic spaces formed as the result of necrosis, numerous phagocytic cells appeared, and new connective tissue was laid down, but subsequently the structure of the transplant was restored by regeneration. With homotransplants the degenerative and inflammatory changes were more marked, and regeneration, if it occurred at all, was long delayed.

Rehn and Lexer (Lexer, 1914, 1919, 1925a) extended the clinical use of adipose tissue transplants and used them with great success not only to fill depressions and cavities but for repairing dural defects associated with injury to the underlying brain, to prevent adhesions about nerves and tendons after freeing these structures from scar tissue and after nerve and tendon suture, and in arthroplasty. They confirmed Verderame's observation that with massive transplants considerable shrinkage occurs, and showed also that multiple small transplants were less likely to survive than a single large one.

Hoesser (1915) used transplants of adipose tissue to repair defects in veins

Binnie (1911) Kanavel (1916) Lewis (1917) and Stewart (1917) published useful reviews of the indications for and the results of the transplantation of adipose tissue in surgery They all confirmed Lexer s finding that such transplants were especially useful for preventing adhesions around nerves and tendons which had been freed from scar tissue or sutured

C B Davis (1917) and Mann (1921) experimented with free autotransplants of omentum Davis reported that such transplants irrespective of their size showed astonishing vitality and persisted with little change whether transplanted subcutaneously or to the peritoneal cavity Mann on the other hand found that omental transplants in the peritoneal cavity were often converted in a few weeks to almost fat free connective tissue although occasionally a small proportion of fat persisted for as long as a year

Loeb (1930) Gurney (1938) and Hausberger (1911) made further studies of the behaviour of autotransplants and homotransplants of adipose tissue in experimental animals Loeb found that homotransplants of adipose tissue in rats and guinea pigs survived longer than homotransplants of thyroid but not as long as homotransplants of cartilage Gurney in an extensive series of experiments in rats found that with autotransplants between a quarter and a half of the transplant survived provided that the tissue was handled gently and care was taken to obtain haemostasis and to avoid infection homotransplants on the other hand were completely destroyed Hausberger also working with rats found that subcutaneous autotransplants of adipose tissue from the vicinity of the testicles of young animals formed lipoma like nodules whereas homotransplants of the same tissue gave rise to nodules which consisted mainly of connective tissue and which began to regress within four months

Moszkowicz (1930) Cotton (1934) Gillies (1934a) Kazanjian and Sturgis (1910) and Stevenson (1949) used free autotransplants of adipose tissue in the treatment of hemiatrophy of the face Cotton in a single case, used many small pieces of gluteal fat Moszkowicz also used multiple transplants but instead of using adipose tissue alone he used strips of fascia lata with the overlying fat attached Kazanjian and Sturgis used a single large transplant in some patients and multiple small transplants in others They found that adipose tissue with attached dermis or deep fascia or both gave good results with either method but transplants of adipose tissue alone were unsatisfactory owing to shrinkage A similar observation was made by Stevenson who used a transplant of adipose tissue alone in one patient with facial hemiatrophy and later had to make a second transplant of adipose tissue and fascia

Neuhof (1941) used a single large autotransplant of adipose tissue to fill a bronchopulmonary cavity in a patient who had had a lung abscess complicated by empyema Despite the gross infection and the presence of several bronchial fistulae the wound healed and the cavity was permanently obliterated

Berson (1944) used free autotransplants from the buttocks consisting of dermis adipose tissue and deep fascia in reconstruction of the breast after loss due to abscess injury or surgical excision and in patients with faulty development of the breasts A similar procedure was used later by Barnes (1953)

Peer in an extensive study of experimental free transplants of adipose tissue in human patients (Peer 1950 Peer and Walker 1951) found that single large autotransplants irrespective of whether or not they were in contact with like tissue were reduced to about half their original weight

and volume in the course of a year, while multiple small autotransplants lost about four fifths of their weight in the same period It was found however that autotransplants sometimes increased in size if the patient became more adipose in respect of the particular fat system from which the graft was taken". Histological observations suggested that in every case the fat cells present in the transplant a year or more after transplantation were "surviving or descendant fat cells from the original graft" and did not originate from neighbouring host tissue or wandering host cells Blood vessels containing red blood cells and leucocytes could be identified in the transplants as early as four days after transplantation. Some of these vessels appeared to belong to the original vascular system of the transplant and to have anastomosed with host vessels.

Homotransplants, in Peer's experiments, when examined after periods ranging from 3 to 13 months, consisted of connective tissue infiltrated with round cells and giant cells, and containing many cystic spaces, with no recognizable adipose tissue.

In the light of these and later studies Peer (1956 concluded that "free autogenous fat grafts with overlying dermis provide the best available grafting material to substitute for soft tissue deficiencies in the cheeks [see Fig 91] and breasts where a soft, even contour is required " He found that the grafts took better if the patient was placed on a fat-free reducing diet before operation

So far, in this section we have been considering only free transplants, but mention must now be made of pedicle transplants. We shall exclude pedicle transplants of subcutaneous tissue and skin used for the repair of surface defects as these have been discussed in Chapter 14.

Jiano (1913) patched a vein with a pedicle transplant of adipose tissue, and Binnie (1914) and others used pedicle transplants of omentum to control haemorrhage from wounds of the liver, and from the gall bladder bed after cholecystectomy; these procedures, however, are now quite obsolete. Binnie also advocated the use of pedicle transplants of adipose tissue in arthroplasty, and to provide cover for tendons and nerves after suturing these structures or freeing them from scar tissue. O'Shaughnessy (1936) investigated the use of pedicle grafts of omentum attached to the heart as a means of treating coronary thrombosis (p. 443), but the procedure has not proved very effective

Gillies (1920a) demonstrated the use of local flaps of subcutaneous adipose tissue based either on the deep fascia or on the skin for restoring the normal contour after excising a depressed scar.

Biesenberger (1931) devised an operation for the treatment of ptosis and hypertrophy of the breasts in which he used a rotation flap fashioned from some of the excess adipose and glandular tissue, and including the nipple and areola, for the reconstruction. Another operation, employing a rotation flap of adipose tissue and dermis, was introduced later by Longacre (1953) for reconstruction of the breast after subtotal or total excision.

Neumann (1953) used pedicle flaps of subcutaneous fat and dermis in the treatment of three patients with hemiatrophy of the face. He first raised an abdominal tubed pedicle graft of skin and subcutaneous tissue, and attached one end to the wrist. One to five months later he detached the tube from the abdomen, removed the epidermis, and introduced the free end of the flap into the subcutaneous tissue of the cheek. Subsequently the graft was freed from the wrist and revision procedures were undertaken to reduce the bulk of the buried part of the flap.

Use in Present Day Surgery

Autologous subcutaneous adipose tissue, with or without attached dermis, deep fascia or both, is used to fill small depressions in any part of the body but especially in the

face, in the treatment of hemiatrophy of the face, in mammaplasty, and to provide cover for nerves and tendons which have been sutured or freed from scar tissue.

It is widely believed that free transplants are very liable to become infected and also to necrose, but according to Peer (1956) these dangers have been exaggerated and about 80 per cent of grafts take well if the technique is satisfactory. In particular asepsis and haemostasis must be scrupulous, the tissue must be handled very gently, and the operation must be completed expeditiously. Even under the most favourable conditions

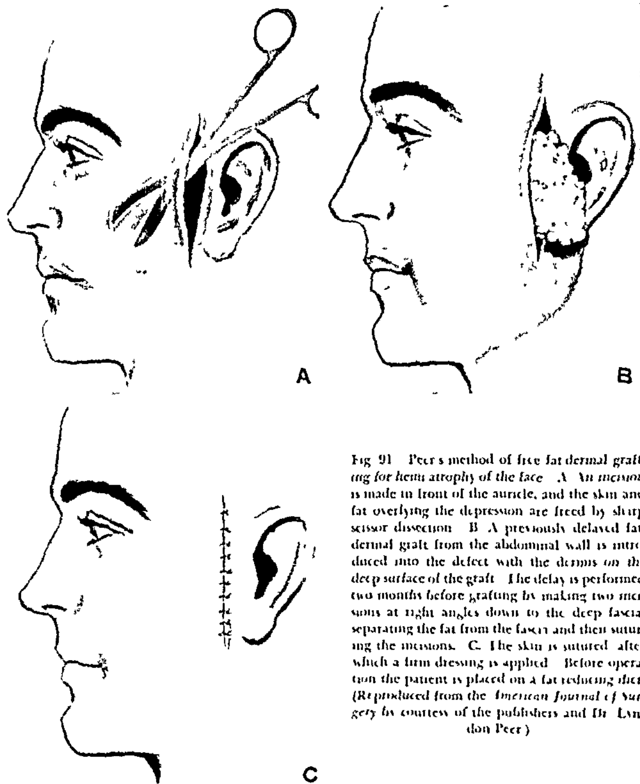


Fig 91 Peer's method of free fat dermal grafting *for hemiatrophy of the face* A *An incision* is made in front of the auricle, and the skin and fat overlying the depression are freed by sharp scissor dissection B A previously delayed fat dermal graft from the abdominal wall is introduced into the defect with the dermis *on the deep surface of the graft* The delay is performed two months *before* grafting by making two incisions at right angles down to the deep fascia separating the fat from the fascia and then suturing the incisions. C. The skin is sutured after which a firm dressing is applied Before operation the patient is placed on a fat reducing diet (*Reproduced from the American Journal of Surgery* by courtesy of the publishers and Dr Lyndon Peer)

some shrinkage is likely to occur in free grafts, and its extent is unpredictable, but the risk of gross shrinkage is probably lessened if the patient is put on a fat-free diet before operation and the overlying dermis is included with the graft.

All these complications are less likely to occur with pedicle transplants. Whenever possible, therefore, local flaps of adipose tissue should be used. When this is not possible one must balance the convenience and simplicity of a free transplant against the relative freedom from complications of a pedicle transplant from some distant site.

Autologous omentum, either in the form of a pedicle transplant or less commonly as a free graft, is used in abdominal surgery to reinforce the suture line after operations on the gastro-intestinal tract; for example after closure of a perforated peptic ulcer, and sometimes after intestinal anastomosis.

Homografts of adipose tissue have no place in present day surgery.

HETEROTOPIC TRANSPLANTATION OF DERMIS AND WHOLE SKIN

History

Dermal transplants have been used in reconstructive surgery for two main purposes: firstly, for the repair of herniae, torn ligaments and severed tendons, and other structures where considerable tensile strength is required, secondly, to fill depressions. It will be convenient to consider these two uses separately.*

The notion of using dermal transplants, in preference to transplants of fascia or tendon, in reconstructive operations in which tensile strength is required, was suggested independently by Loewe (1913) and Rehn (Rehn, 1914a; Rehn and Miyauchi, 1914). Subsequently dermal autotransplants were used experimentally by Rehn (1914b) and Schwartz (1922) to repair the divided tendo Achillis in dogs, and clinically by Rehn for repairing herniae, torn ligaments and severed tendons, but outside Germany little interest was aroused until Uihlein published a review of Rehn's cases in 1939.

The good results obtained have been attributed to the structure and physical properties of dermis, and to the fact that the transplants were sutured under tension and, in consequence, subjected to a functional stimulus. No systematic histological study was undertaken by Rehn or his colleagues, but Uihlein (1939) had occasion to operate on two patients who had received dermal transplants in Rehn's clinic four years previously, and was able to show that the transplants had the structure of normal vascular connective tissue with no recognizable epithelial elements.

Rehn's findings were confirmed by Cannaday and his colleagues (Cannaday, 1942, 1943, 1946), who, in the light of experience of dermal transplants in 129 patients, concluded that dermis "may be used in all cases in which the use of fascia or tendon might be indicated, with the expectation of superior results. It heals in rapidly and well; is strong and stable from the time of operation; has good vitality; is able to survive under adverse conditions; possesses great tensile strength; has a good blood supply; gradually assumes the function of the part it replaces; is gradually converted into fibrous tissue and is readily available when needed." Other surgeons, including Swenson and Harkins (1943), Scola (1944), Harkins (1945), West and Hicks (1948), May and Spann (1948), and Swenson (1950) have also reported good results with dermis

*A helpful general and historical review of the use of dermal transplants has been published by Brown (1951).

grafts in the repair of incisional, recurrent and other herniae. Still more recently *Geever and Merendino* (1952) have reported favourably on the use of free dermal transplants for the repair of large experimental defects of the diaphragm in dogs.

In all these investigations the surface epithelium was carefully removed from the transplants, partly to reduce the risk of infection, but mainly because the work of Schweninger (1881), Reverdin (1887), Garre (1894), Davis and Traut (1926), Zinches (1931), Imakita (1932), Okuma (1936), and others, had shown that—at any rate in experimental animals—buried epidermis or whole skin might give rise to epidermoid cysts. It was conjectured by Mair (1945), however, that cysts *would not develop from transplants of whole skin which were sutured in such a way that they were under tension*. Experiments in rabbits confirmed this conjecture, and Mair proceeded to use buried autotransplants of whole skin clinically in the repair of herniae of various kinds. He reported (Mair, 1945, 1946, 1948) that the results were good and that all epithelial *elements disappeared from the transplants* without giving rise to cysts.

Mair's technique, or a modification of it, has been used successfully by other surgeons including Goodall and Guthrie (1947), Swanker (1948), Chodoff (1949), Greene and Wallgast (1949), Vasquez (1949), Lisele and Starkloff (1951), and Spina and Reginato (1953). On the other hand Gray, Mansberger and Yeager (1951) found in experiments in dogs that buried skin, even when sutured under tension, gave rise to cysts which persisted indefinitely; in addition keratinous debris accumulated on the epithelial surface of the graft preventing union with host tissues. This of course, does not mean that the same sequelae occur in man, indeed Mair's published observations suggest that as a rule they do not. It must be recorded however that the author has seen a patient with a huge epidermoid cyst in the groin following repair of an inguinal hernia by means of buried whole skin. It would seem that the danger of cyst formation could be avoided by using homologous skin but so far there is no record of this having been tried.

The use of buried dermis to fill depressions was initiated by Lexer (1914), who *used dermal autotransplants "for padding* out the tip of the nose and the alae nasi, as well as to ... build up an entire auricle," and by Eitner (1920), who used a dermal autotransplant to fill a subcutaneous depression in the face of a patient who had previously been treated by the injection of paraffin wax. Subsequently, similar transplants were used by Blair (1932), Brown *(1932), Straatsma (1932) and others for the* repair of saddle noses.

Peer and Paddock (1937), fearing that, despite removal of the surface epithelium, epidermoid cysts might develop from hair follicle, sebaceous gland or sweat gland epithelium, made careful histological observations on dermal autotransplants buried beneath the skin of the chest in human *patients*.* *They found that with most* transplants some epithelium remained and formed closed cysts containing horny material and fragments of hairs, these cysts were of microscopic size, however, and the epithelium lining them disappeared after a few months. Sebaceous glands were no longer recognizable after two weeks, and hair follicles disappeared after about *two months*; sweat *glands, on the other hand, could be* recognized even after a year, but by that time were in process of degenerating and being replaced by connective tissue.† In the

*These patients were receiving cartilage autotransplants for the treatment of nasal deformities. Surplus cartilage was stored subcutaneously in case the transplant became infected, and experimental dermal transplants were buried at the same time.

†Essentially similar results were obtained in later experiments in which autotransplants of whole skin were *buried (Peer, 195[illegible]; Peer and Walker, 1951).* It would thus seem that buried skin autotransplants behave differently in man and in experimental animals.

light of these findings Peer and others (Eagleton, Swain and Peer, 1937; Peer, 1940, Schuessler and Steffanoff, 1949) have used dermal autotransplants, with or without accompanying subcutaneous fat and deep fascia, to fill depressions of various kinds in the skull and the face, and Berson (1944), as we have seen, used autotransplants of dermis, subcutaneous fat and deep fascia to fill depressions in other regions including the breasts

Use in Present Day Surgery

Autologous dermis or whole skin is still used by some surgeons in repairing herniae. In the author's view, however, whole skin should never be used owing to the definite, though possibly slight, risk of infection or cyst formation. Dermis is not open to the same objection but there is no convincing evidence that it is superior to either fascia or non-living materials such as tantalum, stainless steel or nylon mesh. Nylon mesh is especially useful. It can be cut to any required size and shape, is easily sutured, and, provided adequate aseptic precautions are taken, gives excellent results.

Buried autotransplants of dermis, with or without subcutaneous fat and deep fascia, are used for filling depressions, especially in the head and neck.

Homotransplants of dermis are rarely if ever required because sufficient autologous tissue is almost always available, and when this is not the case it is better to use either fascia or non-living material (*v. supra*).

TRANSPLANTATION OF FASCIA

History

Transplantation of fascia* was initiated by McArthur (1901, 1904), who used strips of the aponeurosis of the external oblique muscle as living sutures in the repair of inguinal herniae, and Murphy (1904), who used pedicle transplants of fascia in arthroplasty [illegible] in the knee and later in other joints

During the ten years preceding the first world war the scope of fascial transplantation was greatly extended

In the first place the experimental and clinical studies of Kirschner (1909, 1910, 1913) and other surgeons (Gunther, 1910; Rothschild 1910 Hohmeier, 1911a, b, König, 1911 Kostenko and Rubaschew, 1912; Nassetti, 1912, Kornew, 1913; Lexer, 1914) in Europe, and Staige Davis (1911), Lewis and C B Davis (1911), Halsted (1913) and Mann (1914) in America, showed that free transplants of fascia could be used successfully for repairing torn ligaments and tendons, herniae of various kinds, and defects in the pleura, dura mater, diaphragm, trachea and oesophagus; for wrapping around aneurysms; and for building up aponeuroses in order to transfer muscle function. Autotransplants were used almost exclusively, but according to Lexer (1914) it was shown experimentally by Rehn that homotransplants functioned very similarly.

Secondly, autologous fascia was used to an increasing extent in arthroplasty, and good results were reported, especially in arthroplasty of the knee, hip, elbow and temporo-mandibular joints. Murphy (1912a, 1913, 1914a), though aware (Murphy, 1913) that free transplants could be used successfully, continued to use pedicle transplants in most cases, and his example was followed by other American surgeons (Scudder, 1907, 1908; Whitman, 1911; Edmunds, 1912). Some European surgeons also used pedicle transplants, but others, including Putti (1913) in Italy, and Thom (1911), Denk (1912), Exner (1913) and Payr (1914a)

*The term fascia is used here in a wide sense and includes inter alia muscle aponeuroses and serous membranes.

in Germany used free transplants of fascia lata

Thirdly Busch (1910) devised a method of correcting the deformity in patients with permanent facial paralysis by using a buried sling of *aluminium bronze wire** to elevate the angle of the mouth on the paralysed side and subsequently he (Busch 1913) Stein (1913) and Kirschner (1913) used a sling of autologous fascia lata attached below to the tissues at the angle of the mouth and above to the periosteum of the maxilla for the same purpose

During the first world war there were reports of the successful use of fascia in arthroplasty by MacAusland (1915) who used pedicle transplants in two cases and free transplants in another two Ashhurst (1915) Davis (1915) Whitman (1916) McKenna (1917) Ceccarelli (1917) Henderson (1918) and others in the treatment of facial paralysis by Gillies (1917) and in the treatment of visceral defects by Neuhof (1917)

Since then the technique of fascial transplantation has been improved and the indications for this procedure have been clarified

Gallie and Le Mesurier (1921 1924) in the light of extensive experimental studies in rabbits devised a special needle for inserting living fascial sutures and demonstrated the value of such sutures in the treatment of hernia and also though to a lesser extent in the treatment of injuries of tendons and ligaments paralytic deformities and congenital ptosis Subsequently autologous fascia has been used extensively for repairing hernia by many surgeons including Gallie (1932) Wangensteen (1934) McLoskey and Lehman (1940) Joyce (1940) and Smith and Masson (1940) mainly in the form of living sutures but sometimes for large and difficult hernia in the form of a transplanted fascial sheet (Gallie 1932) or a pedicle graft (Wangensteen 1934) and also for repairing ligaments and large tendons notably the lateral and cruciate ligaments of the knee (Groves 1917a 1920 Edwards 1921 Campbell 1935 1936 1939 Carrell 1937) the quadriceps tendon and ligamentum patellae (Gallie and Le Mesurier 1927) and the tendo Achillis (Zadek 1940)

Autologous fascial sutures were used in the operative fixation of fractures by Patterson (1928) in the treatment of dislocation of the acromioclavicular joint by Patterson (1928) and Bunnell (1928b) and to provide intra-articular stabilization in recurrent dislocation of the shoulder by Gratz and Robison (1932)

Cleveland (1933) used fascia to construct a new digital flexor sheath in a patient who also received a free tendon transplant to replace a flexor tendon which had been destroyed by infection

The technique of treating facial paralysis by fascial slings was improved by Blair (1926 1930) who used a modified Reverdin needle to insert the fascia and anchored each sling to paralysed muscle below and to the temporal fascia above Reports of successful cases were published by Moszkowicz (1928) Fischer (1929) Lodge (1930) Sheehan (1932b) Brooke (1933) Wardill (1933) Gillies (1934b) Brown (1939) and others

Fascial slings provide satisfactory correction when the face is at rest provided that as Blair (1926) emphasized the deformity is at first over-corrected To obtain reanimation of the face Gillies (1934b) turned down a strip of temporal muscle and then used a piece of fascia as a bridge between this strip of muscle and the tissue at the angle of the mouth and this procedure was subsequently modified by Bunnell (1937) Brown (1939) and Owens (1947) achieved much the same result by using fascial slings attached below to the angle of the mouth and anchored above in the substance of the temporal muscle An alternative approach

* [illegible] have been used [illegible] for the same purpose by Schuessler (1940) and [illegible]

[illegible] the problem [illegible] the female [illegible] in some [illegible] fascia [illegible] described in Chapter 11.

[illegible] recommended the use of fascia [illegible] to compensate for [illegible] anatomical [illegible] He attached the [illegible] in [illegible] the pubis [illegible] later [illegible] 1917 [illegible] pubis [illegible] the [illegible]

[illegible]

[illegible] have also been used in the treatment [illegible]

[illegible] incontinence [illegible] urethral and external [illegible] passed [illegible] sutured the [illegible] incontinence [illegible] by passing [illegible] around the urethra [illegible] and anchoring the [illegible] rectus muscle on each side.

These operations, although quite different in detail, [illegible] Aldridge (1942), [illegible] of the previous work, [illegible] patients for stress incontinence which combined repair of the urethra perhaps by the usual vaginal approach with the insertion of two pedicled strips of external oblique aponeurosis. He attached them by their medial ends to the pubis, passed retropubically around the proximal urethra, and sutured together from above and again. Studdiford (1955) reported favourably on this procedure but subsequently (Studdiford, 1956, 1958) modified it by substituting a single long strip of external rectus sheath, passed around the urethra and anchored to the rectus sheath on the opposite side, for the two strips used by Aldridge. Millin and Read (Millin, 1947; Millin and Read, 1948) modified the procedure for use in patients in whom incontinence had persisted after an adequate vaginal operation for prolapse. They used two pedicled strips of rectus sheath and passed them around the proximal part of the urethra after exposing it by a retropubic approach. An operation differing only slightly from that of Millin and Read was developed independently by Marshall ([illegible]), and subsequently he (Marshall, [illegible]) performed a similar operation with preserved ox fascia in place of autogenous fascia. Yet another operation has been described by Shaw (1949a, b), in which a free graft of fascia lata is used. The urethra and neck of the bladder are exposed by a vaginal approach and the centre of the graft is applied to these structures and anchored to surrounding tissue. The ends of the graft are then passed upwards on each side through incisions in the pubococcygeus muscle. The pubic bone on each side is exposed through a small incision in the [illegible] and the ends of the graft are anchored by passing them through holes drilled in the bone.

The use of fascial slings for the treatment of anal incontinence was initiated by Wreden of Leningrad in 1929. Wreden passed two loops of fascia lata [illegible] from each side circumferentially around the anus, interlocked them, and anchored the ends of each sling to fibres of the gluteus maximus muscle. Shortly afterward Stone (1929) reported the successful use of [illegible]

two in which he performed a similar operation using preserved fascia and Ransohoff (1929) and Price (1933) each reported a successful result after using Wreden's original technique. This operation is applicable only when the patient has no anal sphincter or when his sphincter is permanently paralysed. In such cases however better results can now be obtained by using a gracilis muscle transplant (p. 316) and Wreden's operation has become obsolete.

The technique of using free transplants of fascia lata in arthroplasty was refined and popularized by Putti (1920, 1921), MacAusland (MacAusland 1921, MacAusland and MacAusland 1929), Campbell (1921, 1924, 1925, 1932, 1939), Hey Groves (1923), Albee (1931), Hark (1944), Ryerson (1944), Speed and Knight (1944), Fowler (1947) and other surgeons; the use of pedicle transplants of fascia, on the other hand, was gradually abandoned.

For many years the joints regarded as most suitable for arthroplasty by interposition of fascia were the temporo-mandibular joint (MacAusland and MacAusland 1929), the elbow (MacAusland 1921, Ryerson 1944), the knee (Putti 1920, Campbell 1921, Hark 1944) and the hip (Campbell 1924, Speed and Knight 1944). Nowadays, owing to the high proportion of unsatisfactory results, arthroplasty is rarely performed in the knee; arthroplasty of the hip is performed by the vitallium cup technique (Smith-Petersen 1939, 1948) or by insertion of a femoral head prosthesis,* and in arthroplasty of the temporo-mandibular joint it is often considered unnecessary to insert fascia. On the other hand good results have been obtained in arthroplasty of the metacarpo-phalangeal joints of the fingers by interposition of fascia (Fowler, 1947).

Bailey and his colleagues (Bailey, Jamison, Bakst, Bolton, Nichols and Gemeinhardt 1954) attempted to treat mitral incompetence by means of free and pedicled transplants of *pericardium*. They expressed the opinion that it would soon be possible to obtain just as satisfactory results in patients with mitral incompetence as those obtained by valvulotomy in patients with mitral stenosis, but this prediction has not yet been fulfilled.

So far we have been considering autotransplantation of living fascia. Koontz (1926, 1927) used heterotransplants of alcohol-preserved fascia, first in experimental animals and later clinically for the repair of herniae. Haas (1931a) showed experimentally that preserved dead fascia united with muscle as quickly and as firmly as living fascia; it evoked a more marked inflammatory reaction, however, and did not maintain its elasticity as well as fresh autologous fascia. Weinberg (1938) used preserved heterologous (ox) fascia to repair tendon defects in dogs with good results, and Koontz and Shackleford (1941), also working with dogs, reported favourably on the use of preserved fascia for suturing experimental fractures of the olecranon.

Use in Present Day Surgery

Living autologous fascia is used for the following purposes:

1. The repair of herniae.
2. The repair of tendons and ligaments.
3. The treatment of facial paralysis and ptosis, and occasionally other forms of paralysis.
4. The treatment of stress incontinence of women.
5. The treatment of cerebrospinal rhinorrhoea associated with fractures of the base of the skull.
6. Arthroplasty.

Fascia lata, which is most often used, may be obtained by open operation or by using a fascia stripper as shown in Figure 92. By using a stripper a long incision is avoided

* The res[illegible] of the techniques mentioned are often [illegible] and at present there is no [illegible] on the [illegible] arthroplasty of the hip.

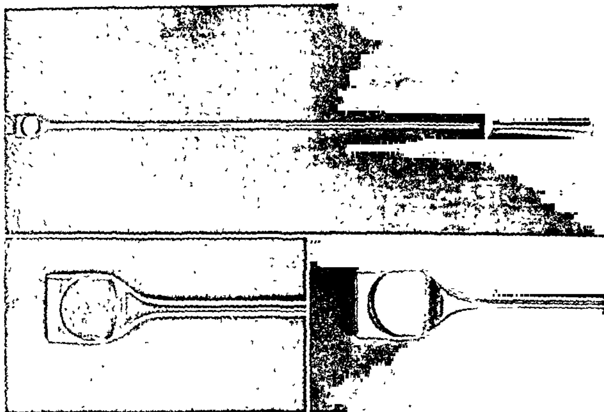


Fig 92 Fascia stripper The whole instrument is shown above, and the front and back of the distal end are shown below on a larger scale To obtain fascia lata a short transverse incision is made in the distal part of the thigh and a strip of fascia about half an inch wide is dissected up for a short distance. The free end is threaded between the blades of the stripper and the strip is further dissected by passing the stripper upwards subcutaneously The upper end of the strip is then divided subcutaneously by means of a tenotomy knife, and instrument and fascia are withdrawn.

Some surgeons state that muscle hernia occurs more frequently after stripping than after open operation but it is doubtful whether this is true

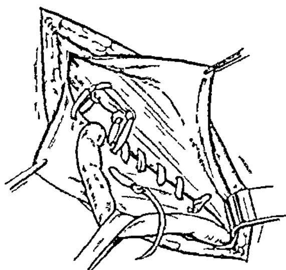
Preserved homologous and heterologous fascia is rarely used because sufficient autologous tissue is usually available, and when this is not the case, implants of nylon, stainless steel or tantalum mesh, can often be used instead

Repair of Herniae

In direct inguinal hernia, and in cases of indirect inguinal hernia associated with weakness of the posterior wall of the inguinal canal predisposing to the development of a direct hernia, fascia may be used to fill the space between the conjoined tendon and Poupart's ligament. Most commonly sutures of fascia lata, inserted with a Gallie needle, are used to form a latticework (Fig 93). Strips of external oblique aponeurosis are occasionally used instead, but it is usually impossible to obtain sufficient of this material. Alternatively, a free transplant of fascia lata may be used in the form of a patch, or a pedicle transplant of rectus sheath or fascia lata may be swung into the defect.

Fascia lata is used also in repairing incisional herniae when reconstruction with local tissue is impossible. Fascial sutures are used as a rule but a patch may be used instead. In either case the transplanted fascia must be securely anchored to strong aponeurotic tissue at the margins of the defect.

Non-living materials such as stainless



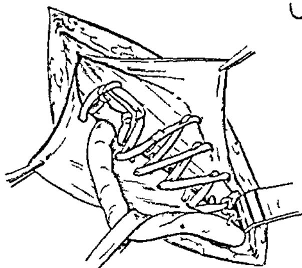


Fig. 33. Method of using sutures of living fascia lata in inguinal herniorrhaphy described by Gallie and Le Mesurier. The first layer of sutures in this procedure (shown on left) approximates the conjoined tendon to Poupart's ligament. The second layer is inserted loosely and is attached to the external oblique aponeurosis above and to Poupart's ligament below. (Redrawn from the *British Journal of Surgery* by courtesy of the publishers and Dr W. E. Gallie.)

steel, tantalum and nylon mesh, and floss silk may be used instead of fascia in repairing herniae. They have the merit of convenience and give good results when asepsis is adequate, but are troublesome if infection occurs.

The author's preference is for nylon mesh, especially in the repair of large incisional herniae, but fascia is still used in many clinics.

Repair of Tendons and Ligaments

Living sutures of autologous fascia lata are used for repairing the quadriceps tendon and ligamentum patellae, and the tendo Achillis in cases of neglected rupture of these structures. Free fascial transplants are used also for repairing other ligaments, including the acromio-clavicular ligaments and the orbicular ligament of the radius (see Mayer, 1952).

Pedicled strips of fascia lata are used for reconstructing the internal and external lateral ligaments of the knee, and the anterior cruciate ligament. The internal lateral ligament may alternatively be repaired with a pedicled strip of fascia from the medial side of the knee. The various techniques which are employed are described in detail by Campbell (1919).

Treatment of Facial Paralysis and Congenital Ptosis

There are three main types of operation for the treatment of facial paralysis: provision of static support by inserting slings of either autologous fascia or metal; re-animation of the face by pedicle muscle transplants; and re-innervation of the paralysed muscles by nerve suture, nerve anastomosis or nerve grafting. In long-standing cases associated with atrophy of the facial muscles the choice lies between providing static support by fascia or metal, re-animation by pedicle muscle transplants, or some combination of these procedures. Here we shall consider the use of fascial slings; pedicle transplantation of muscle and nerve grafting will however be discussed in Chapters 16 and 17 respectively.

Strips of fascia lata about 0.5 cm. wide and as long as possible are obtained with a stripper. A curved incision is made over the temporal muscle and a Blair needle is

passed subcutaneously from this incision to the middle of the lower lip where a small incision is made below the mucocutaneous junction. One end of a strip of fascia is grasped with the needle, which is then withdrawn so that the fascia emerges through the temporal incision. The middle of the fascial strip is anchored to the orbicularis oris and the other end is then brought up to the temporal region. Two more slings, anchored below to the orbicularis oris at the middle of the upper lip and at the angle of the mouth respectively, are inserted in the same way and the upper ends of all three are anchored to the temporal fascia under sufficient tension to over-correct the deformity (Fig. 91). A fourth strip may be looped around the angle of the mouth from the middle of the upper lip to the middle of the lower lip, and, if the upper part of the face is paralysed as well as the lower, a fifth strip is inserted in the lower eyelid, and anchored at one end to the inner canthus and at the other to the temporal fascia.

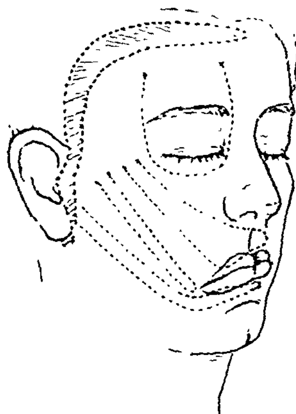


Fig. 91. Blair's (1926) method of treating facial paralysis by inserting fascial strips. The shaded area denotes redundant skin which Blair was accustomed to excise in appropriate cases. The dotted lines other than the line forming the boundary of the shaded area denote the positions of the fascial strips. (*Reproduced from Annals of Surgery by courtesy of the publishers.*)

Various operations have been devised for the treatment of ptosis. One method, which is especially useful in patients with complete lesions of the oculomotor nerve, is to insert a loop of fascia lata subcutaneously with a Blair needle and anchor it below to the tarsal plate, and above to the frontalis muscle.

Treatment of Stress Incontinence

Stress incontinence is commonly associated with uterine and vaginal prolapse, and if it is of sufficient severity to warrant surgical treatment some form of vaginal operation is performed to correct the prolapse.

When stress incontinence persists after an adequate vaginal operation of this kind a fascial sling operation by the technique of either Millin, Marshall or Shaw (p. 288) offers good prospect of curing the patient. Marshall (1948) has suggested that these operations might be performed as primary procedures in patients with stress incontinence without any associated prolapse, but most gynaecologists do not accept this view.

When the prolapse recurs and stress incontinence persists after a vaginal operation, the procedures of Aldridge or Studdiford may be used.

The indications for, and technique of, these various operations are discussed in detail by Read (1950).

Treatment of Cerebrospinal Rhinorrhoea

In post-traumatic cerebrospinal rhinorrhoea the defect in the dura may be closed with a free transplant of fascia lata. After turning down a frontal flap the defect is located and the graft inserted by either an extradural or an intradural approach. The technique is described in detail by Rowbotham (1942).

Arthroplasty

Arthroplasty by interposing fascia after separating and reshaping the articular ends of the bones entering the joint is performed in the elbow and sometimes in the metacarpo phalangeal joints of the fingers by the techniques already described (p 286)

It is nowadays seldom performed in other joints In the hip other methods are used such as vitallium cup arthroplasty or insertion of a Judet or other type of prosthesis In the temporo mandibular joint excision of part of the condyle of the mandible combined with reshaping to form a new condyle and excavation of a suitable cavity in the temporal bone usually suffices without insertion of fascia In other joints including the ankle knee wrist and shoulder and the metacarpo phalangeal joint of the thumb arthrodesis in the optimal position is usually preferable to arthroplasty

TRANSPLANTATION OF TENDON

Tendon transplantation in its widest sense includes free transplantation tendon transfer and tenodesis

Free transplantation denotes transplantation of an isolated portion of tendon It may be orthotopic in which case the transplant is attached above to tendon and below to either tendon or bone or heterotopic as for example when the transplant is used to form a pulley for another tendon

Tendon transfer implies that a tendon arising from a functioning muscle is divided near its insertion and then attached either to the distal stump of another tendon or to bone Tendon transfer is sometimes referred to as pedicle transplantation It is important to note however that although the tendon remains connected to the muscle from which it arises its blood supply may be almost completely interrupted during the process of transfer

Tenodesis means using a tendon as a ligament by giving it a new attachment to some appropriate bony point with the object of stabilizing a joint As a rule the tendon of a paralysed muscle is used but there are exceptions to this The tendon may or may not be severed from its muscle belly

The evolution of tendon transplantation has been profoundly influenced by increasing knowledge of the processes of regeneration and repair in tendon and by developments in the technique of tendon suture Before discussing experimental and clinical observations on tendon transplantation therefore it will be convenient to consider briefly the history of these related topics

REGENERATION AND REPAIR IN TENDON

The healing of ruptured tendons was studied by John Hunter (1786) who observed similarities between this process and the healing of a fracture It was not until the nineteenth century however that interest in this subject became widespread

Von Ammon (1837) stated that a defect in a tendon was first filled by a formless exudate and that later new tendon regenerated from the tendon stumps Paget (1858) claimed however that the exudate was replaced by undifferentiated fibrous tissue which subsequently assumed the structure of tendon

The function of the exudate was much debated Billroth (1858) and Adams (1851 1860 1869) held that the exudate delayed healing Pirogoff (1840) Viering (1891) and others that without it regeneration could not occur

There was also sharp difference of opinion concerning the origin of the cells respon

sible for the repair. Güterbock (1872) held that they originated mainly from surrounding connective tissue and tendon sheath; Beltzour (1883) that the tendon stumps played an important role. Viering (1891), in the light of experiments on the healing of the divided tendo Achillis in rabbits, claimed that peritendinous or intratendinous connective tissue, as distinct from specialized tendon cells, were primarily responsible. Enderlen (1893), however, after studying repair of the tendo Achillis in guinea pigs, concluded that specialized tendon cells, cells of the peritendineum internum and externum, and to some extent also surrounding connective tissue cells, all played a part, and further evidence in support of this view was obtained later by Borst (1903), Seggel (1903), Minervini (1907) and Sever (1911).

The influence of functional activity on the healing of tendon has been widely studied. Viering (1891) observed that functional stimulation influenced the orientation of cells in healing tendon. Rehn (1919) showed that tendon grafts retrogressed unless subjected to function. He showed further, and Schwarz (1922) and Hueck (1923) confirmed, that non-specialized connective tissue transplanted into a tendon defect gradually assumed a tendon-like structure if it was subjected to a functional stimulus. Lange (1929) showed that if a silk thread was left between the divided ends of a tendon and after healing constant gentle traction was maintained on the thread, in a direction perpendicular to the axis of the tendon, new connective tissue was laid down in the direction of the thread and subsequently assumed the structure of tendon. Biesin (1931) concluded that tendon healed primarily by non-specialized connective tissue which, under the influence of tension and use, became differentiated into tendon or tendon-like tissue.

Another problem which has attracted much attention is the manner in which healing of a tendon is affected by the presence of a synovial sheath. Where there is no such sheath a tendon is surrounded by specialized tissue which permits gliding to occur and to which Biesalski and Mayer (1916) gave the name paratenon,* and it was found by many observers that tendon surrounded by paratenon healed much more satisfactorily than tendon enclosed in a synovial sheath. To account for this Bier (1917) and Salomon (1924) postulated that there was a substance in synovial fluid which inhibited regeneration. In addition Salomon held that the synovial membrane covering a tendon was much inferior to paratenon as a source of proliferating cells. He suggested that regeneration would occur more readily if the sheath were left widely open, but the experiments of Hueck (1923) failed to confirm this prediction.

Schwarz (1922) and Hauck (1924) stressed the importance of a good blood supply for healing. It is commonly held (*see* Edwards, 1946) that vessels approach the paratenon from the tissues and pass through it at irregular intervals to reach the surface of the tendon. Recent investigations in rabbits (Skoog and Persson, 1954; Nisbet, personal communication) and man (Clark, 1956) suggest however that non-synovial tendons possess two vascular coverings (the epitenon and the paratenon) separated by a potential space, and that vessels do not traverse this space except at one point on the circumference where the paratenon dips in to form a structure very like the mesotenon of synovial tendons. It seems likely therefore that, contrary to what is commonly believed, non-synovial tendons have no advantage over synovial tendons as far as their blood supply is concerned.

Mason and his colleagues have studied the time relations of the healing process. Mason and Shearon (1932) found that initially union was effected by proliferation of

*Previously this tissue was referred to as peritendineum externum.

connective tissue on and about the tendon, but that after four or five days specialized tendon cells begin to proliferate and to enter into the formation of the tendon callus This stage of repair, they found, lasted about two weeks, and was followed by a stage in which the junctional tissue becomes further differentiated into tendon or tendon-like tissue. Subsequently Mason and Allen (1941) made serial measurements of the tensile strength of sutured tendons, as a result of which they distinguished three phases in the healing process These are, firstly, a phase lasting about five days in which the tensile strength rapidly diminishes, secondly, a phase during which the tensile strength gradually increases until a maximum is reached about the sixteenth day, and thirdly a phase, beginning between the nineteenth and twenty first days and continuing for an undetermined period, during which there is a further increase in tensile strength. These phases correspond histologically to a phase of exudation and fibrinous union, a phase of fibroplasia, and a phase of maturation, respectively Function, according to Mason and Allen, is not beneficial and may be harmful during the first two phases, but during the third phase it accelerates the gain of tensile strength

Carstam (1953) showed that in rabbits administration of cortisone reduced the amount of adhesion formation around tendons which had been scarified Berkin (1954) showed further that cortisone in similar dosage (5 mg per kg body weight per day) did not impair the healing of rabbit tendons following division and suture, as judged by histological examination and tests of tensile strength He suggested, therefore, that cortisone might reasonably be tried clinically to discourage adhesion formation following repair of tendons

THE TECHNIQUE OF TENDON SUTURE

Repair of tendons by suture is mentioned in the writings of Ambroise Paré (1510 1590), but little progress could be made in this branch of surgery until the advent of aseptic methods

The modern technique of tendon suture is largely the creation of Bunnell (1918, 1922, 1924, 1928a, 1948) and Mayer (1916, 1936, 1938), though some important contributions have been made by other surgeons including Rischai (1910), Harmer (1921, 1922), Koch and Mason (Koch and Mason, 1933, Mason, 1936, 1940, Koch, 1943, 1944), and Pulvertaft (1948) The essentials of the technique have been well summarized by Koch and Mason (1933) and Bunnell (1948) They include general anaesthesia, adequate exposure by means of incisions placed as far as possible in crease lines, or along the lateral borders of the fingers, scrupulous asepsis, a bloodless field, obtained by using some form of tourniquet, great gentleness in handling all tissues, accurate apposition, and post operative splinting until union is sufficiently strong, after which active exercises are begun Primary suture may be performed with relatively clean wounds seen within four to six hours of injury, in other cases, however, it is usually better to postpone suture of the tendon until the skin wound has healed

The suture materials which are generally used are silk and stainless steel wire It is usually stated that catgut should not be used because it evokes a much more intense reaction than the other materials mentioned (O Shea, 1937), but this question merits further investigation

Different methods of suturing are illustrated in Figures 95 97 Figure 95 shows Bunnell s method of suturing a round tendon with silk The silk remains permanently *in situ* Stainless steel wire may be used in the same way as advocated by Pulvertaft (1948) Figure 96 shows another method devised by Bunnell (1940, 1944, 1948) in which stainless steel wire is used, but instead of being left *in situ* permanently is removed

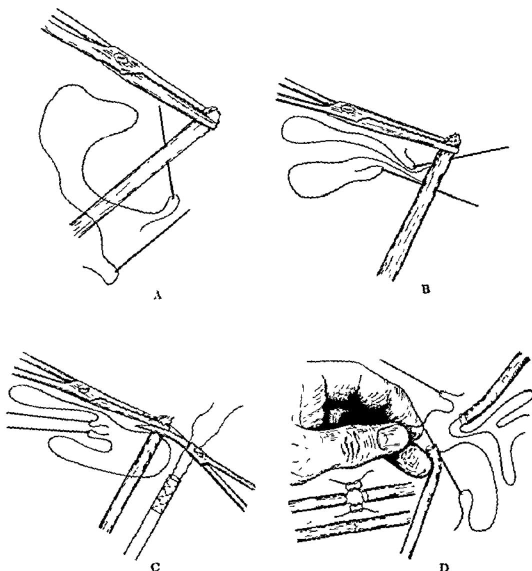


Fig 95 Bunnell's technique for suturing a round tendon with silk. The end of each stump is held in a haemostat while the suture is being inserted (A, B) and the crushed tissue is then excised (C) The sutures are knotted between the tendon ends (D) (Reproduced from the *Journal of Bone and Joint Surgery* by courtesy of the publishers)

after about three weeks by means of a pull-out wire A simple method of attaching a tendon to bone is to pass it through a hole drilled in the bone and retain it in place with a stainless steel wire until union has occurred According to Kernwein (1912) union is hastened if the end of the tendon is shredded before it is passed through the bone.

FREE TRANSPLANTATION OF TENDON

History

Gluck (1881) appears to have been the first to demonstrate experimentally that tendon could be freely transplanted and survive. He transplanted the gastrocnemius muscle and tendo Achillis homologously in fowls and reported that the tendon appeared macroscopically normal after being *in situ* for periods up to forty days

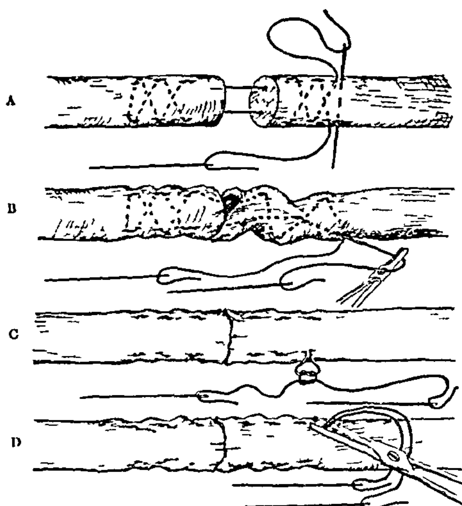


Fig. 96. Bunnell's method of suturing a round tendon with stainless steel wire. A. Placing the stitch. B. All slack is removed by pulling on one wire at a time. C. A single knot, which sinks into the tendon when it is tied, is used remote from the suture line. D. The ends of the wire are cut off after again being passed through the tendon so that they will be left embedded in its substance. (Reproduced from the *Journal of Bone and Joint Surgery* by courtesy of the publishers.)

A few years later Peyrot (1887) and Monod (1887) each reported a clinical case in which a free tendon heterotransplant was used. In Peyrot's case a segment of dog tendon 1 cm. long was used to fill a defect in a digital flexor tendon. Despite the fact that the wound failed to heal by first intention, the transplant remained in place, and function, though not perfectly restored, was much improved. In Monod's case a segment of rabbit tendon 5 cm. long was transplanted to a defect in the flexor pollicis longus tendon, and function was perfectly restored.

It soon became apparent that the results described in the preceding paragraph were exceptional, and that much experimental work would have to be undertaken if free tendon transplantation were to become an acceptable clinical procedure.

The problem was attacked on both sides of the Atlantic, notably by Kirschner, Rehn, Lexer and Schwarz in Germany, and by Lewis, Davis, Mayer, Bunnell and Mason in the United States.

Kirschner (1909) showed that autotransplants survived but lost their characteristic structure unless subjected to functional stimulation by permitting movement at an

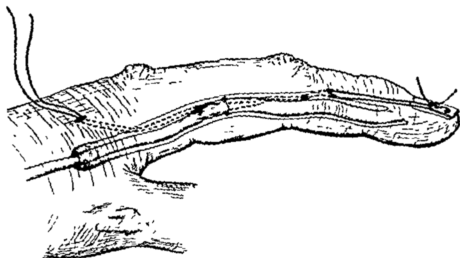


Fig 97 Bunnell's method of repairing a tendon with a removable stitch of stainless steel wire The removable stitch is made fast into the proximal stump of tendon and then anchored to the outside of the limb, in this case through the fingernail The tendon ends are approximated with tiny sutures of silk or fine stainless steel wire A pull-out wire is passed through the proximal loop of the removable stitch and brought out through the skin. The removable stitch is withdrawn after about three weeks (Reproduced from the *Journal of Bone and Joint Surgery* by courtesy of the publishers)

early stage. Rehn (1910, 1919) concluded that both autotransplants and homotransplants survived, in both cases however function was successfully restored only if the peritendineum externum (i e. paratenon) of the graft was preserved and movement was started at an early stage. The importance of the peritendineum externum was emphasized also by Schwarz (1922)

Lewis and Davis (1911) studied the behaviour of homologous orthotopic, and autologous heterotopic, transplants of the tendo Achillis in dogs

The orthotopic transplants after seven days were surrounded by granulation tissue, and after three weeks they were fusiform in shape and two or three times as thick as the normal tendo Achillis. Lewis and Davis attributed this thickening partly to proliferation of cells of the peritendineum externum and the surrounding host tissue, and partly to oedema of the transplant. After 59 days the cellular mantle around the transplant was greatly reduced in size and the transplant itself had "practically the appearance of normal tendon," though it was a little thicker than normal. The functional results were excellent provided that the splint was removed early—usually after a week—and the animal was allowed to use the limb. Garlock (1927), and Mason and Shearon (1932), confirmed these findings except that they found it advantageous to wait longer than Lewis and Davis before allowing movement.

The heterotopic transplants in the experiments of Lewis and Davis were placed in pockets in the subcutaneous fat of the abdominal wall. They remained alive but became progressively smaller, and there was no proliferation of the peritendineum. Lewis and Davis attributed these findings, at least in part, to lack of functional stimulus.

Nageotte (1917b, 1919a) studied the behaviour of orthotopic transplants of tendon which had been rendered non-viable by being stored in alcohol. He obtained excellent functional results with both homotransplants and heterotransplants.

While this experimental work was in progress important clinical investigations were also undertaken.

Lexer (1912b, 1914) reported that either autotransplants or homotransplants of tendon could be used successfully for repairing torn ligaments. Subsequently transplants of fascia lata have usually been preferred for this purpose, but autologous tendon has been used, for example, in Henderson's (1930) operation for recurrent dislocation of the shoulder,* and heterologous preserved kangaroo tendon has been used in the repair of herniae (Andrews, 1928) and at one time enjoyed a considerable vogue in orthopaedic surgery.

Orthotopic tendon transplants have been used much more extensively. According to Lexer (1912b, 1914) they should be autologous because adhesions are then less likely to form. Nageotte and Sencert (1918a, b, c), however, found that excellent results could be obtained even with heterotransplants provided that these had been rendered non-viable by being stored in 70 per cent alcohol, and later reports from French and Italian surgeons have supported this claim (Auvray, 1919; Jalifier, 1920a; Regoli, 1922; Nageotte, 1939). Despite this work the great majority of surgeons in other countries have remained sceptical concerning the merits of homotransplants and heterotransplants and have used fresh autotransplants almost exclusively.

Lexer (1912b, 1914) as a rule used the palmaris longus tendon or one of the extensor tendons of the toes. He emphasized the importance of excising all scar tissue before inserting the transplant.

Rehn (1913b) and Dean Lewis (1917) demonstrated the importance of functional stimulation and advised allowing early active movement. Lewis also advised that if an injured tendon was exposed it should be covered with a pedicle graft of skin and fat before tendon transplantation was attempted, and in addition he drew attention to the fact that the results of repair by suture were not as good with flexor tendons as with extensor tendons. It is this fact, or to be more precise the poor results following suture of flexor tendons within the digital sheaths, which has determined the main indication for free tendon transplantation.

The essentials of the modern technique of tendon transplantation in the hand were established within a few years of the end of the first world war by Mayer (1921a, b) and Bunnell (1922). Since then there have been modifications in the technique of tendon suture (p. 295) and, as surgeons have taken a keener interest in this branch of surgery, there has been a marked improvement in the results of transplantation (Garlock, 1926; Harmer, 1926; Bunnell, 1928a, 1948; Graham, 1947; Kinmonth, 1947; Littler, 1947; Pulvertaft, 1948; Boyes, 1950; Rank and Wakefield, 1950, 1951, 1952, 1953; Mayer, 1952; Kyle and Eyre-Brook, 1954).

When gross scarring has occurred after injury of a tendon within a synovial sheath it is necessary before inserting a transplant to excise the scar tissue. The problem of reconstructing a suitable tendon sheath in these circumstances has stimulated much research. Auchincloss (1929) tried using a portion of saphenous vein as a sheath but found that it fused with the tendon. Mayer and Ransohoff (1936a, b) obtained good results in injuries of the digital flexor tendons by inserting a tube of celloidin from the proximal stump of the tendon to the profundus insertion after excising the scar tissue and leaving the tube *in situ* for between four and six weeks. When the tube was withdrawn it left a tunnel with smooth glistening walls along which the transplant was passed. More recently Thatcher (1939) has used stainless steel rods instead of celloidin tubes for reconstructing digital flexor

*In this operation a free autotransplant of the peroneus longus tendon is used to form an extra-articular sling between the head of the humerus and the acromion.

sheaths, and Mayer (1940) has used the original celloidin tube technique for reconstructing the sheath of the extensor digitorum communis.

Use in Present Day Surgery

Indications

Free tendon transplantation finds its main application in reconstructive surgery of the hand. The indications may be considered under two main headings: division of flexor tendons within the digital sheaths, and division of tendons in any situation when, owing to loss of tendon or retraction, the ends cannot be brought together satisfactorily.

Division of Flexor Tendons of the Fingers within the Digital Sheaths. When both the profundus and sublimis tendons to one of the four fingers are divided within the digital sheath proximal to the sublimis insertion, it is usually best to suture the skin without attempting to repair the tendons, and after four to six weeks excise the sublimis tendon and replace part of the profundus tendon with a free transplant of such a length that there is no suture line between the metacarpo-phalangeal and distal interphalangeal joints. Excision of the sublimis tendon and suture of the profundus is sometimes undertaken as a primary or "delayed primary" procedure, especially in children. Free transplantation is not ordinarily performed as a primary procedure owing to the danger of infection, but Rank and Wakefield (1953) have suggested that eventually it may be used as a primary procedure in favourable cases.

When the profundus tendon alone is divided it may pay to accept the disability. Alternatively the profundus tendon may be repaired by suture or replaced by a free transplant, depending on the nature and level of the injury and the age of the patient. If a transplant is used the sublimis tendon may be left in place or removed according to the degree of scarring and consequent interference with function. Free transplantation may also be required when primary repair of a profundus tendon has failed to restore function owing to adhesions.

When the flexor pollicis longus tendon is divided within the digital sheath it may be excised and replaced by a graft of another tendon. Alternatively the distal part of the tendon may be excised and the proximal part slid distally at its junction with the muscle belly sufficiently to enable it to be attached to the base of the distal phalanx.

Division of Tendons in Any Situation When the Ends Cannot Be Approximated Satisfactorily. When, owing to loss of tendon or retraction, the ends of a divided tendon cannot be brought into apposition the choice lies between accepting the disability, inserting a free transplant, or performing tendon transfer. Free transplantation is usually indicated under these conditions when the defect is situated in one of the tendons of the flexor digitorum profundus distal to the metacarpo-phalangeal joint, or when several tendons of the extensor digitorum communis are affected; on the other hand tendon transfer may be the procedure of choice when the defect is situated in the extensor pollicis longus tendon or in one of the tendons of the flexor digitorum profundus proximal to the metacarpo-phalangeal joint.

Technique

In our present state of knowledge only fresh autotransplants should be used. The most generally useful donor tendons are the palmaris longus tendon when present* and the tendons of the long extensor of the toes. These are surrounded by well developed paratenon which can be transplanted with the tendon, and being of small cross section are unlikely to undergo central necrosis

*The palmaris longus is present on both sides in approximately 70 per cent of people.



Fig 98 Flap approach to the digital flexor tendons
(after Rank and Wakefield)

when used as free transplants. To avoid damaging the paratenon the transplant is obtained through an extensive longitudinal incision Alternatively a tendon of the flexor digitorum sublimis, the extensor indicis proprius tendon or the plantaris tendon is used When a sublimis tendon is sacrificed to facilitate repair of the corresponding profundus tendon with a transplant it is tempting to use the sublimis tendon as the transplant The sublimis tendon, however, is devoid of paratenon, and in addition is often too thick, so that as a rule it should not be used

It will suffice to describe free transplantation to the profundus tendon for division of both flexor tendons within the digital sheath The operation is performed under general anaesthesia and a pneumatic tourniquet is used to produce a bloodless field. The flexor tendons in the finger are often approached through a mid lateral incision (Bunnell, 1918, Pulvertaft, 1948), but much better exposure is obtained by raising a flap of skin and subcutaneous fat (Fig 98) as advised by Rank and Wakefield (1952, 1953) The tendons are exposed in the palm through an incision in, or slightly proximal but parallel to, the proximal palmar crease, and both tendons are excised distal to this incision except for the last quarter inch of the profundus tendon This excision should be done by careful dissection rather than by using a tendon stripper The digital flexor sheath* is excised except for two pulleys, one over the proximal and the other over the middle phalanx, and in some cases even these are sacrificed and replaced by new pulleys fashioned from loops of tendon The channel between the finger and palm is enlarged if necessary with Hegar's dilators, and the transplant is then drawn through from the palm to the base of the finger, and finally through the pulleys over the proximal and middle phalanges The distal

* The term digital flexor sheath includes the fibrous as well as the synovial sheath For practical purposes, as Rank and Wakefield (1953) point out, these are "one and indivisible"

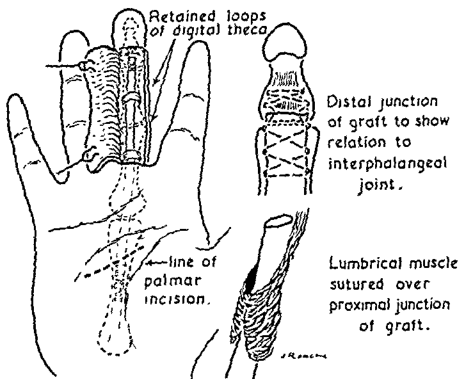


Fig 99 Free tendon grafting for injury of flexor tendons within the digital sheath (Reproduced from *Surgery of Repair as Applied to Hand Injuries*, by courtesy of E & S Livingstone Ltd and Mr. B. K. Rank.)

junction is made first, the transplant being sutured to the stump of the profundus tendon† just distal to the distal interphalangeal joint (Fig. 99) and the digital incision is closed The finger is then held in exaggerated flexion and the proximal stump of the profundus tendon is drawn down with a traction suture while the proximal junction is made in the palm This junction is placed close to the origin of the lumbrical muscle, and when complete first the paratenon of the transplant and then the lumbrical muscle is used to cover the suture line. The tension on the transplant should be such that when the junctions have been made the finger lies "in the correct relationship to the other fingers while under anaesthesia, i e the index finger flexed least and the little finger flexed most" (Pulvertaft, 1948).

† Alternatively the transplant may be attached to the distal phalanx, but this is unnecessary and may be harmful.

Both junctions may be made with retained sutures of silk or stainless steel wire; alternatively, Bunnell's pull-out technique may be used for the proximal junction (Bunnell, 1940, 1941; Kinmonth, 1947). Watson (1955), in making the proximal junction, cuts a V in the profundus tendon, places the end of the transplant in the V, and anchors it with interrupted sutures of braided stainless steel wire.

After closing the palmar incision dressings and splints are applied. Most surgeons (*see* Kinmonth, 1947, Bunnell, 1948; Rank and Wakefield, 1953, Watson, 1955) maintain absolute immobilization for two to three weeks and then allow intermittent active movements within a limited range under supervision, finally dispensing with all splinting after four to five weeks; Pulvertaft (1948), however, allows movement after only six days.

TENDON TRANSFER

History

The first surgeon to perform tendon transfer appears to have been Missa (1770), who successfully united the distal part of a divided extensor tendon of the middle finger to the extensor tendon of the ring finger. Tendon transfer continued to be used occasionally in traumatic surgery during the nineteenth century, and was advocated by Velpeau (1839) and Malgaigni (1862).

Tendon transfer was used for the first time in the treatment of a paralytic disability by Nicoladoni (1882), who attached the peroneal tendons to the tendo Achillis in a patient with paralysis of the calf muscle due to poliomyelitis. Within a few years many operations of this type, in which muscular power is transferred by uniting an active to a paralysed tendon, were performed by German (Drobnick, 1896; Vulpius, 1898, 1904, 1907, Vulpius and Stoffel, 1911), American (Goldthwaite, 1895, Milliken, 1896, Coolidge, 1901), and British (Eve, 1898, Tubby, 1901, White, 1901) surgeons. Several different techniques were used. Most commonly the active tendon was divided completely, re-orientated, and attached to the paralysed tendon near its insertion. Vulpius divided the paralysed tendon well proximal to its insertion, re-orientated the distal portion, and attached the proximal end of the distal portion to an active tendon. Drobnick in some cases split an active tendon and attached part of it to a paralysed one, and Milliken devised a method of "partial and reciprocal grafting" of active and paralysed tendons. It was emphasized by Eve and others that deformity should be corrected prior to tendon transfer, and that the tendon transferred should arise from a muscle whose action was akin to that of the paralysed muscle.

As an alternative to attaching tendon to tendon Drobnick (1896) suggested and practised dividing an active tendon and transferring it to a new insertion by attaching it directly to periosteum. This method was used subsequently by Tubby (1898) in the operation he devised for the treatment of spastic paralysis affecting the forearm and hand, and by Lange (1903, 1907, 1911), Jones (1908), Biesalski (1910) and others in the treatment of paralysis due to poliomyelitis. Wolff (1902), Mueller (1903), Bernstein (1922) and others carried this development a stage further and attached the transferred tendon to bone.

Gluck (1902) tried unsuccessfully to extend with strands of catgut a tendon which was too short to be transferred effectively. Subsequently Lange (1902a, b, 1903, 1907, 1911, 1927) used strands of silk* for this purpose, attaching them distally to periosteum or bone in the vicinity of the insertion of the paralysed tendon. Dense fibrous tissue formed around the silk, and was regarded by Lange as true tendon, but others denied this (Henze and Mayer, 1914). Lange obtained better results in patients with sufficient subcutaneous fat to provide a cover for the artificial tendon than in thin patients. Sever (1912), in the light of experiments using Lange's technique in cats, concluded that the sheath of the transferred tendon, and peritendinous tissue, should be preserved, and that movements should be started early. Rich (1913) reported that clinically Lange's technique gave good results in the shoulder and elbow regions, but not in the lower extremity.

It was soon found that tendon transfer often failed because adhesions formed between the transferred tendon and the surrounding tissue. Biesalski (1910) claimed that this was much less likely to occur if the active tendon was made to traverse the emptied sheath of the paralysed one, and this technique was subsequently widely used by Biesalski himself and Mayer in the treat-

* According to Sever (1912) Anger had attempted to construct artificial tendons of silk as long ago as 1875.

ment of paralytic talipes (Biesalski and Mayer, 1916; Mayer, 1916, 1918, 1921b). Steindler (1918a), who also used this technique, made a point of preserving the mesotendineum of the transposed tendon, but recognised that to do this necessarily restricted the application of the operation. Henze and Mayer (1914) used Biesalski's technique in conjunction with Lange's procedure of extending the transposed tendon with silk, because they had found in using Lange's original method that adhesions occurred not to the silk but to the end of the transposed tendon.

Bernstein (1919, 1922) confirmed that a transposed tendon deprived of its own gliding mechanism was less likely to form adhesions when passed along the sheath of the tendon it replaced, as in Biesalski's method, than when it was surrounded by subcutaneous tissue He obtained even better results, however, by transposing the tendon with its own paratenon or tendon sheath

In addition to the rule that care should be taken whenever possible to maintain a gliding mechanism by one of the methods described above, several other general principles to be observed in performing tendon transfer have been enumerated by Mayer (1916, 1918, 1937, 1952), Dunn (1929, 1937), Ober (1933) and others. There is general agreement that deformity should be corrected prior to, or at the latest at the same time as, the tendon transfer; that the transferred tendon should be under the proper amount of tension, and its line of pull should be such that the system is mechanically efficient, and that the muscle giving rise to the tendon which is transferred should be sufficiently powerful to replace the paralysed muscle, or that failing this additional tendons should be transferred or bone stabilizing operations should be performed The muscle which motivates the transferred tendon should, generally speaking, have an action akin to that of the muscle it replaces; this rule is however not always observed in the upper extremity where, as Dunn (1929) has pointed out, re-education of an individual muscle to act apart from its group is more feasible than in the lower extremity.* It has also long been agreed by most surgeons that the transferred tendon should be attached to bone, although an ingenious method of tendon to tendon junction, in which either the active or the paralysed tendon is used as a living suture, was devised and used by Royle (1924, 1928).

Use in Present Day Surgery

Upper Extremity

In the upper extremity tendon transfer† is used in the treatment of irrecoverable radial paralysis, to provide opposition of the thumb in patients with paralysis of the median nerve or its thenar branch, and less commonly to improve function in patients with a variety of other disabilities due to nerve lesions. It is also used occasionally in the treatment of severed tendons.

Radial Paralysis. Tendon transfer was used first in the treatment of radial paralysis by Murphy (1914b), who transferred the flexor carpi radialis to the extensors of the fingers and thumb in two patients. The procedure was modified and popularized by Robert Jones (1916a, 1917, 1921), Stoffel (1918), McMurray (1919), and Starr (1922); and more recently further modifications have been introduced and studied by many surgeons including Steindler (1939, 1940), Abbott (1944), Zachary (1946), Young and Lowe (1947), Luckey and McPherson (1947), and Bunnell (1948). The operations which have been most often performed are summarized in Table II.

*To avoid repetition we shall defer consideration of the extent to which re-education can occur, and the nature of the process, until we discuss muscle transfer in Chapter 16 (p. 308).

†Sometimes much of the fleshy part of an active muscle, and not merely its tendon, is shifted with the object of replacing a paralysed muscle. This procedure, which will be referred to as muscle transfer, will be considered in Chapter 16.

TABLE II

TENDON TRANSFERS FOR RADIAL PARALYSIS

Author and Date	Finger Extensors		Thumb		Wrist	Remarks
	Index	Others	Long Extensor	Abductor with or without Short Extensor	Radial Extensor	
Murphy (1914b)	F C R	F C R	F C R			
Jones (1916a, 1917, 1921)	F C R	F C U	F C R		P T	
Stoffel (1918)	F C U	F C U	F C U	F Subl to ring finger	F C R	
Starr (1922)	F C R F C R	F C R F C R	P L F C R	P L	P T	Conserves one wrist flexor (F C U)
Steindler (1940)	F C U	F C U	F C R	P L		
Abbott (1944)	F C R	F C U	F C R		P T	Adds arthrodesis of wrist
Zachary (1946) Young & Lowe (1947)	various combinations					Advise conserving one wrist flexor if possible
Luckey & Mc[illegible] (1947)	[illegible]	[illegible]	[illegible]	P T [illegible]	P T	If wrist unstable add arthro- [illegible]
Bunnell (194[illegible])	[illegible] F C R F C R	[illegible] F C U F C R	[illegible] F C R F C R	[illegible] P T via E C R L P T via E C R L	[illegible] P T P T	[illegible] wrist flexor

ABBREVIATIONS (after Zachary)

Pronator teres	P T
Flexor carpi radialis	F C R
Flexor carpi ulnaris	F C U
Palmaris longus	P L
Abductor pollicis longus	A P L
Extensor carpi radialis longus	E C R L
Flexor digitorum sublimis	F Subl

Bunnell, who has contributed greatly to this as to other aspects of the surgery of the hand, has pointed out that what is required is power to extend the proximal joints of the fingers and thumb, combined with stability of the wrist in both dorsal and volar flexion and stability of the base of the thumb. To maintain stability of the wrist in volar flexion Bunnell, like Starr and Zachary, advises that one wrist flexor should be conserved whenever possible, and when this is not possible he advises slitting the retinaculum of the abductor pollicis longus so that this tendon when activated bows forward to such an extent that it acts as a wrist flexor. When insufficient tendons are available to fulfil all the desiderata enumerated the wrist joint should be arthrodesed, thus allowing the tendons which would otherwise be used to stabilize the wrist to be used for other purposes.

Loss of Opposition of the Thumb. Several procedures have been devised for the treatment of this disability.

In Ney's (1921) operation the tendon of the extensor pollicis brevis is divided at the wrist and freed to its insertion. The proxi-

mal end of the distal portion is then passed upwards below the flexor retinaculum and attached to the flexor carpi radialis tendon or to the proximal part of the divided palmaris longus tendon.

Steindler (1918b, 1939, 1940) split the flexor pollicis longus tendon, detached the radial half and, after passing it in a spiral manner around the radial side of the thumb, attached it to the dorsum of the proximal phalanx.

Lyle (1924) has combined the operations of Ney and Steindler.

Bunnell (1924, 1938, 1948) maintained that the tendon used to obtain opposition should be inserted on the dorso-ulnar aspect of the base of the proximal phalanx of the thumb, and should pass subcutaneously from its insertion in the direction of the pisiform bone

Other Disabilities Due to Nerve Lesions. To activate the profundus tendons to the index and ring fingers in patients with median nerve paralysis Bunnell (1948) used the profundus tendons to the other two fingers, reinforcing them if necessary with the brachioradialis In irrecoverable ulnar nerve lesions he improved the muscle balance in the clawed fingers by detaching the sublimis tendons from their insertions and re attaching them to the transverse fibres and lateral bands of the corresponding dorsal extensor expansions, and provided for abduction of the index finger by attaching the extensor indicis proprius tendon to the tendon of the first dorsal interosseous muscle (Bunnell, 1942, 1948).

Various other operations, some of them combining tendon transfer with arthrodesis, have been devised for use in patients with lesions of more than one nerve or of the brachial plexus (Green, 1942; Luckey and McPherson, 1947; Bunnell, 1948).

Severed Tendons. Tendon transfer, though originally introduced for the treatment of severed tendons, is not performed very often for this purpose today. It is however sometimes useful, notably after division of a flexor digitorum profundus tendon in the palm, when the corresponding sublimis tendon may be transferred, and after division of the extensor pollicis longus tendon, when the common extensor tendon to the index finger may be used (Bunnell, 1948; Rank and Wakefield, 1953).

Lower Extremity

In correcting deformities in the lower extremity stability is the primary consideration and this is most commonly achieved by arthrodesis. Tendon transfer has a place, however, notably in the treatment of paralytic talipes.

Paralytic Talipes. Tendon transfer has been widely used in the treatment of paralytic talipes (Tubby, 1901; Mayer, 1921b, 1924, 1929, 1937, 1952; Bernstein, 1922; Dunn, 1929, 1937; Williamson, 1932; Ober, 1933; Peabody, 1938; Carpenter, 1939), and although the indications have narrowed since the development of bone stabilizing operations by Hoke (1921), Dunn (1922), Ryerson (1923) and others, it is still of value either alone or in combination with a stabilizing operation when there is what Peabody (1938) terms *dynamic imbalance* of the foot Valgus deformity due to paralysis of the tibialis anterior, for example, may be treated by triple arthrodesis combined with transfer of the peroneus longus tendon and tenodesis of the proximal end of the distal part of this tendon; paralytic varus by transfer of the tibialis anterior; paralytic calcaneus by triple arthrodesis combined with transfer of the peronei, the tibialis posterior and the flexor digitorum longus, or some of these tendons, to the tendo Achillis; and paralytic drop foot by a stabilizing operation combined with transfer of the peroneus longus to the site of insertion of the tibialis anterior and the peroneus brevis to an insertion at the base of the fourth metatarsal (Mayer, 1952).

Other Conditions. Tendon transfer has been used successfully to correct clawing of the great toe due to poliomyelitis and other causes (Forrester Brown, 1938), and by Garceau (1940) in the treatment of congenital talipes when varus deformity has recurred after other forms of treatment

TENODESIS

History

Tenodesis means giving a tendon a new bony attachment in order to make it function as a ligament

Tenodesis, using the tendon of a paralysed muscle, was advocated in poliomyelitis by Tilanus (1898), Codivilla (1900) and Vulpius (1912), and this procedure was developed subsequently by Gallie (1913, 1916, 1921) and others (*see* Jones, 1914, Dunn, 1929) Originally Gallie (1913) used a whole tendon in the treatment of paralytic varus, valgus and calcaneus deformities. Later he (Gallie, 1916) devised an operation for the treatment of paralytic calcaneus in which he split the tendo Achillis longitudinally, divided half the tendon, and anchored the proximal end of the distal portion to the tibia about two inches above the ankle joint. Dunn (1929) has commented favourably on this operation

Royal Whitman (1921) devised an operation for the treatment of equino valgus deformity due to paralysis of the tibialis anterior in which the peroneus brevis and tertius tendons are divided sewn together, passed through the emptied sheath of the paralysed tibialis anterior, and fixed to the inner border of the foot, after which the tendon of tibialis anterior is looped round the extensor tendons of the toes and fixed to a groove cut in the base of the medial malleolus * This loop operation, as it is termed, was subsequently modified by Royal Whitman's son Armitage (Whitman, 1931) and combined with arthrodesis of the subastragaloid and astragalo-scaphoid joints.

*In addition the tendon of flexor hallucis longus is cut and its proximal part is attached to the inner border of the foot

Nicola (1929, 1934) devised an ingenious operation for the treatment of recurrent dislocation of the shoulder which is essentially a tenodesis of the tendon of origin of the long head of the biceps brachii The tendon is exposed by splitting the deltoid and the capsule of the shoulder, and divided one inch distal to the bicipital groove. The proximal portion is then drawn downwards through a hole drilled from the floor of the bicipital groove to the articular surface of the humerus, re united to the distal portion, and also stitched to the ligaments and periosteum of the bicipital groove As might be expected from the results of animal experiments in which tendon has been inserted in holes drilled in bone (Kernwein, Fahey and Garrison, 1938), the biceps tendon becomes firmly united to the humerus after Nicola's operation

Bunnell (1948) introduced a modification of his operation to restore opposition of the thumb in which the flexor tendon which is joined to the tendon of the extensor pollicis brevis is not passed through a pulley but is attached to the ulna about an inch and a half above the wrist The thumb will then be made to oppose whenever the wrist is dorsiflexed This modification is used when there are *insufficient active muscles* for the original operation to be effective, provided that dorsiflexion of the wrist is possible

Use in Present Day Surgery

Tenodesis is still used occasionally in combination with other types of operation in the treatment of paralytic talipes Nicola's operation has had a considerable vogue, and has yielded excellent results in some hands, but is now rarely used The modified Bunnell operation for restoring opposition of the thumb by tenodesis is occasionally indicated

CHAPTER 16

Transplantation of Voluntary Muscle

Voluntary muscle, if it is to function effectively and retain its structural integrity, must be innervated, in addition it must possess an adequate blood supply, and its skeletal connexions must be such that it is able to contract when stimulated In discussing muscle transplantation a distinction must therefore be drawn between muscle transfer, in which these criteria are fulfilled, and heterotopic transplantation of muscle in which they are not fulfilled.

MUSCLE TRANSFER

The term muscle transfer will be used to denote a form of transplantation which fulfils the following conditions:

1 The whole or portion of the fleshy part of a muscle is transplanted autologously with an adequate blood supply, and with its nerve supply intact

2 The insertion of the transplant is shifted but its origin remains the same, or *vice versa*

3 The transplant is able to function in its new site, and to compensate, to some extent at least, for the disability caused by paralysis, injury or imperfect development of some other muscle

The distinction between muscle transfer and tendon transfer is not always clear cut; we shall, however, use the term muscle transfer when the operation involves shifting a considerable bulk of muscle tissue, irrespective of whether the change of insertion is effected by joining one tendon to another or not.

Generally speaking the conditions for successful tendon transfer discussed in Chapter 15 apply also to muscle transfer, except that unless tendon is transferred with the muscle the need for providing a gliding mechanism does not arise. In particular, static deformity must be corrected, the transferred muscle must be sufficiently powerful to perform its new function, and its line of pull must be such that the system is mechanically efficient. It is futile to transfer part of a muscle and expect the two parts to perform opposite functions; on the other hand it is often impracticable, and fortunately not essential, to use a muscle having a similar action to the one it replaces, because in man a considerable degree of re-education is possible.

EXPERIMENTAL BACKGROUND

Though much remains unknown, some insight into the nature of the re-education process may be gained by comparing the results of muscle (and tendon) transfer in man and lower animals.

Despite earlier views to the contrary, Sperry (1939, 1940, 1942) has shown that in rats no adaptive adjustment occurs when a flexor muscle is transferred to the position of an extensor or *vice versa*, or when a flexor

and an extensor are transferred reciprocally, in either the upper or the lower extremity, apart from the development of certain trick movements. In cats Watrous and Olmsted (1940) found, as Anochin and Chernevskiy (1935) had reported earlier, that co-ordinated movement was restored after replacing a flexor muscle in the lower extremity by an extensor or *vice versa*, and they made similar observations also in dogs. They found however that when the animals were made decerebrate, and the transferred muscles were isolated and prepared for kymographic recording, their reflex activity was the same as if transfer had not been performed. It seems therefore that in these instances either there was no adjustment and the action of the transferred muscle was masked by the action of other muscles, or else adjustment occurred at a very high level in the central nervous system.

It seems clear that in man co-ordination of movement after reciprocal transfer of flexor and extensor groups could be achieved by conscious mental effort; thus after transfer of the flexors and extensors of the ankle for example, it would merely be necessary, as Sperry (1945) has pointed out, for the patient to make a mental effort to flex the foot when he wished actually to extend it and *vice versa*. In practice, however, reciprocal transfer such as this is never performed, instead, one or more muscles are used to replace others which are paralysed. There has been much discussion as to whether, when one of a group of muscles is transferred, it can be made to contract independently of other members of the group, and the available records, though numerous, are either not sufficiently detailed or too inconsistent to enable a final conclusion to be drawn. According to Scheib (1927, 1928a, b, 1938) who has made what is probably the most thorough study of the problem, as far as the muscles of the upper extremity are concerned a great deal can be achieved in this direction by diligent practice. The extent to which deliberative corrections can become rapid, automatic and generalized, so as to transfer readily to unpractised activities', as Sperry (1945) has remarked, can however "only be guessed at present."

The experiments so far considered refer to transfer of limb muscles (or tendons). Transfer of ocular muscles appears, at least at first sight, to present special features, and to provide evidence of radical and rapid central nervous re-organization. It seems likely, however, that the findings in rabbits, dogs, cats, and other species apart from primates, may be accounted for by the action of the retractor bulbi muscle, which itself can provide correct eye movements in all directions (*see* Dusser de Barenne and de Kleyn 1928, Watrous and Olmsted, 1940, Sperry, 1945). The observations of Marina (1912, 1915), who transferred ocular muscles in monkeys and found that normal co-ordinated eye movements in both voluntary and automatic reactions were restored in three to four days, cannot be explained in this way because monkeys have no retractor bulbi muscle. The same holds good for the more recent observations of Leinfelder and Black (1941, 1942), which in large measure confirm, and also extend, those of Marina. These investigators found in Rhesus monkeys that co-ordinated eye movements were restored* as early as eight days after transferring reciprocally the medial and inferior recti and the superior and lateral recti, leaving the two oblique muscles in their normal positions, and that the degree and speed of recovery were not appreciably influenced by keeping the animals in complete darkness during the recovery period. Co-ordinated movements persisted if the superior oblique muscle was subsequently divided, but failed to develop if this muscle

*The eye movements were not entirely perfect, but the functional disturbances were trivial in comparison with what would have been expected from the anatomical disarrangement.

was divided at the original operation. Sperry (1945) has suggested that all these results might conceivably be due to the formation of adhesions between the tendons of the transferred muscles themselves and extraneous tendon connexions to points near their original insertions; at present however the whole matter must be regarded as unsettled.

The problem we have been considering resembles in some ways the problem of re-education after nerve crossing (p. 342). In muscle or tendon transfer, however, the motor and proprioceptive innervation of the muscle remain unchanged, whereas after nerve crossing the situation is more complex and less well defined. It is clear that the motor units of the muscle become connected with foreign lower motor neurons but little is known about the extent to which proprioceptive re-innervation occurs or the pattern, if any, which it follows (Sanders, 1942).

MUSCLE TRANSFER IN CLINICAL PRACTICE

In considering muscle transfer in clinical practice it will be convenient to arrange the discussion under the following headings:

1. Muscle transfer for paralysis of limb muscles.
2. Muscle transfer for facial paralysis.
3. Transfer of the extra-ocular muscles.
4. Muscle transfer for sphincter lesions.

Muscle Transfer* for Paralysis of Limb Muscles

History

In the superior extremity muscle transfer operations have been devised for the treatment of paralysis of the supinators, the flexors of the elbow, the biceps, the deltoid, the supraspinatus, the external rotators of the shoulder, and the serratus anterior.

Muscle transfer for paralysis of the supinators of the forearm was first performed by Tubby (1899, 1912), who detached the pronator teres from its insertion, passed the muscle through the interosseous membrane, and re-attached it to the outer surface of the radius. This procedure, either alone or combined with transfer of the flexor carpi radialis in the same manner, enjoyed a considerable vogue, but according to Steindler (1939, 1944) it is preferable to transfer the flexor carpi ulnaris via a subcutaneous tunnel to a new insertion into the dorsum of the radius.

For paralysis of the flexors of the elbow Stoffel (1918) advocated transfer of part of the triceps to the flexor aspect of the elbow, but it is clearly unreasonable to expect part of the triceps to act as a flexor and part as an extensor. It is theoretically sounder and, as Bunnell (1948) has shown, it is satisfactory in practice, to detach the whole triceps insertion from the olecranon, extend the muscle with a free graft of fascia lata, and re-insert it to the tuberosity of the radius. A simpler procedure devised by Steindler (1939), which has been used extensively, is to transfer the common origin of the flexors of the wrist and fingers from the internal epicondyle to a higher position on the humerus. Another alternative was devised by Clark (1946), who fashioned a *pedicled strip from the pectoralis major* and attached it to the tendon of the biceps close to the radial tuberosity.

For paralysis of the triceps Ober and Barr (Ober and Barr, 1938; Ober, 1944) shifted the belly of the brachioradialis to a site overlying the triceps and olecranon without detaching either its origin or its insertion. Other surgeons have made little use of this operation, however, and its value is open to question.

Transfer of part of the trapezius for paralysis of the deltoid, according to Bunnell (1948), was first performed by Stoffel in 1921, but the operation was developed by Mayer (1927, 1939) and Haas (1935). In Mayer's technique the insertion of the upper

*Excluding tendon transfer (Chapter 15)

half of the trapezius is detached from the clavicle and the spine of the scapula, prolonged with a free graft of fascia lata, and fastened to the humerus in the vicinity of the deltoid insertion with the arm abducted 60°. An alternative procedure was devised by Ober (1932, 1941), who detached the short head of the biceps and the long head of the triceps from their origins and attached them to the acromion, and reserved transfer of part of the trapezius for the treatment of paralysis of the supraspinatus. These operations are not often indicated because in paralysis of the abductors of the shoulder it is usually best to arthrodese the joint, but according to Mayer (1939) they should be used when the condition is bilateral in preference to arthrodesis of both shoulder joints.

For paralysis of the external rotators of the shoulder due to birth palsy the usual procedure, if operative treatment is undertaken, is tenotomy of the pectoralis major and subscapularis tendons, and sometimes also of the tendon of origin of the coracobrachialis and short head of the biceps. L'Episcopo (1934) added a further procedure, namely transfer of the insertion of the teres major from the bicipital groove to the posterior aspect of the shaft of the humerus.

For paralysis of the serratus anterior Haas (1934) detached the pectoralis major from its insertion, passed the muscle through the triangular space, and attached it with silk sutures to the angle of the scapula. Ober (1941) and Durman (1945) modified this procedure by transferring only the lower third of the muscle and prolonging it by means of a free graft of fascia lata.

In the inferior extremity muscle transfer has been used mainly in the treatment of paralysis of the quadriceps femoris and the gluteus medius muscles.

According to Dunn (1928) transfer of the sartorius muscle was first suggested for paralysis of the quadriceps femoris by Goldthwaite. The procedure was modified by Mayer (1921b), who transferred the biceps femoris together with either the sartorius, the semitendinosus or the gracilis; Dunn and Stuart (1924), who transferred the tensor fasciae femoris together with a strip of the ilio-tibial band; and Ober (1933), who transferred the sartorius and the tensor fasciae femoris. In these operations the transferred muscle was given a new insertion either to the quadriceps tendon or directly to the patella.

Muscle transfer for paralysis of the gluteus medius was initiated by Legg (1923), who transferred the tensor fasciae femoris with some fascia lata to a new insertion into the outer surface of the shaft of the femur two to three inches below the trochanter. Later, Legg (1933) devised an alternative operation in which he used the same muscle but transferred its origin to a more posterior position on the iliac crest. Telson (1928) transferred the lateral half of the gluteus maximus instead of the tensor fasciae, giving it a new origin from the anterior part of the iliac crest. Wagner and Rizzo (1936), in patients with paralysis of the gluteus medius associated with weakness of the gluteus maximus, detached the tensor fasciae femoris, the sartorius and the straight head of the rectus femoris from their origins, sutured them together, passed them through a tunnel under the paralysed gluteus medius and the anterior border of gluteus maximus, and reattached them to the ilium in the vicinity of the posterior inferior spine.

Use in Present Day Surgery

Many of the operations discussed in the preceding section are seldom performed now, but, with the exception of transfer of part of the triceps for paralysis of the elbow flexors, none can be regarded as completely obsolete. The ones which have become most widely accepted are Tubby's and Steindler's operations for supinator paralysis, and Steindler's operation for paralysis of the elbow flexors.

Muscle Transfer for Facial Paralysis

History*

According to Halle (1938), muscle transfer was first used in the treatment of facial paralysis by Lexer in 1908, following a suggestion by Wrede. Lexer used two pedicled strips from the anterior half of the masseter muscle, attaching one to the muscle of the upper and the other to the muscle of the lower lip, and two strips from the temporal muscle, attaching one to the upper and one to the lower eyelid.

Various other procedures, similar in principle but differing in detail, were soon introduced. Gomoiu (1908) and J. Jianu (1909) attached a pedicled strip of sternomastoid muscle to the lip commissure. A. Jianu (1909) split the masseter and attached part to the muscle of the upper and part to that of the lower lip. Eden (1911) attached a pedicled strip from the masseter to the angle of the mouth and another strip from the temporal muscle to the outer canthus of the eye. Morestin (1915b), in a single case, attached the buccinator muscle to the anterior edge, aponeurosis, and superficial muscle fibres of the masseter.

Gersuny (1916) introduced a different principle and transplanted paralysed muscle into normal muscle in the hope that the transplanted muscle would become innervated from the normal muscle. He reported some improvement in function.

Fenwick (1919) originally used a technique similar to that of Eden (*v supra*), but found it difficult to fashion the strip from the masseter. Later, therefore, he used two strips from the temporal muscle, attaching one to the orbicularis oris and one to the orbicularis oculi. Hastings (1920), in a single case, split the masseter, and then detached half of the muscle from the jaw and re-attached it with catgut sutures to the tissue at the angle of the mouth. Perthes (1924) and Schmid (1938) reported favourably on cases treated by Lexer's method. Adson (1925) reported one good result in two cases treated by Eden's method, and Pickerill (1928) also commented favourably on this procedure.

Brunner (1926) introduced a new technique in which he prepared a pedicled transplant from the masseter by an intraoral approach, thus avoiding an external scar.

Sheehan (1932a, 1935), like Fenwick (1919), used two strips from the temporal muscle, attaching one to the orbicularis oculi and the other to the muscle at the angle of the mouth. To facilitate the action of the strip attached to the angle of the mouth he pared down the zygoma. Sheehan (1935) claimed that the paralysed muscles to which the strips were attached became re-animated.

Gillies (1934b), and later Bunnell (1937), fashioned pedicled strips from the temporal muscle and the masseter, and then prolonged them with free grafts of fascia lata.

Halle (1938) used two strips from the masseter, one large and one small. The small strip he attached to the levator anguli oris; the larger he divided into two parts, and attached these to the orbicularis oris in the upper and lower lips respectively. Alexander (1941) used this technique successfully in one case.

Adams (1946) devised an operation in which he used strips from the masseter, the temporal and the frontalis muscles, and attached them to the lips, the eyelids and inner canthus, and the eyebrow region respectively. He reported that the transplanted muscle did not show active contraction for four to six weeks.

McLaughlin (1953) devised yet another operation in which he detached the temporal muscle from its insertion into the coronoid process of the mandible by an intra-oral approach and connected it by means of a subcutaneous sling of fascia lata to the angle of the mouth.

*Much of the information in this section has been obtained from a review by Owens (1951).

Use in Present Day Surgery

In cases of facial paralysis in which it is not feasible to reinnervate the paralysed muscles by nerve suture or grafting (p 326) the choice of treatment lies between the insertion of fascial slings muscle transfer, or some combination of these procedures

Fascial slings alone as we have seen (Chapter 15) give static support and if anchored to healthy muscle may provide some degree of reanimation Muscle transfer combined when necessary with the insertion of bridges of fascia as in the operations of Gillies (1934b) Bunnell (1937) and McLaughlin (1953) offers the possibility of a greater degree of reanimation but is technically more difficult to perform and to be successful requires intelligent co operation from the patient Given an experienced surgeon and a suitable patient it is the procedure of choice

Transfer of the Extra ocular Muscles*

History

Muscle transfer was first used in ophthalmic surgery for the treatment of ptosis After various investigators (*see* Jackson 1923) had tried to correct this condition by connecting the upper lid to the frontalis muscle with catgut silk gold wire or chain kangaroo tendon and other materials Darier (1897) and Fergus (1901) developed the technique of transferring slips from this muscle and attaching them either to the skin of the lid or to the tarsus These operations had some measure of success but a more effective procedure was devised by Motais (1897) who split the tendon of the superior rectus parallel to its fibres by two incisions detached the central portion of the tendon from its insertion and reattached it to the tarsus and skin of the upper eyelid Motais operation was subsequently used successfully by O Connor (1921) Bruns (1922) Jackson (1923) and others A modification was introduced by Arfinquez (1920) who divided the tendon of the levator palpebrae superioris and attached the distal stump to the undivided superior rectus tendon

Jackson (1903) transferred the insertion of the superior rectus posteriorly and laterally to compensate for paralysis of the superior oblique muscle and later (Jackson 1923) reported that the results of this operation have been satisfactory and permanent

Hummelsheim (1907 1908) after experimental trial in monkeys split the tendons of the superior and inferior recti in a twelve year old girl with congenital paralysis of the external rectus and attached the temporal portion of each to the tendon of the paralysed muscle The patient eventually obtained 30° of abduction Good results following this operation have also been reported by Woodruff (1917) Sweet (1921) and others Hummelsheim s original technique has been extended or modified in various ways Stuelp (1912) added tenotomy of the internal rectus O Connor (1919 1921) detached the tendon of the external rectus and split it into three segments shortened the middle segment, and attached the upper and lower segments to slips from the superior and inferior recti respectively Peter (1936) combined transfer of part of the tendons of the superior and inferior recti with advancement of the paralysed external rectus and recession of the internal rectus

McDannald (1914) treated a patient with congenital absence of the inferior rectus by tenotomy of the superior rectus combined with transfer of part of the tendons of the internal and external recti to the paralysed tendon the patient was said to have been more comfortable afterwards though the operation does not appear to have been an outstanding success A similar case was reported subsequently by Posey (1921)

*It should be observed that recession to diminish the action of an overactive muscle and advancement or insertion to enhance the action of one which is insufficiently active do not fall within the scope of our definition of muscle transfer

O'Connor performed an analogous operation for cosmetic reasons in a patient with paralysis of the superior rectus associated with blindness in the same eye due to a gunshot wound of the orbit.

Jackson (1923) described two alternative operations for complete paralysis of all the muscles supplied by the oculomotor nerve, which he had worked out on the cadaver but had not used in a living patient. In the first he divided the tendon of the superior oblique, withdrew the proximal stump through the pulley, and sutured it to the tendon of the paralysed internal rectus. In the second he split the tendon of the external rectus, detached the upper portion, and re-inserted it in the vicinity of the superior rectus insertion. Jackson felt that in a young patient "a limited field of binocular fixation and stereoscopic vision" might be re-established, but recognized that the annoyance from diplopia might be increased if the image in the deviating eye were "brought close to or upon the macula, but not so that it could fuse perfectly with that of the fixing eye." The second of these operations was modified by Wiener (1928), who combined advancement and resection of the internal rectus with transfer of the external rectus insertion to a position beneath the insertion of the superior rectus. The first has been modified by Peter (1936, 1938), who advocated performing recession of the external rectus at the time of the muscle transfer and later shortening the levator palpebrae superioris.

Use in Present Day Surgery

O'Connor's operation (p. 313), or Peter's modification of it, is used in selected cases of complete lateral rectus paralysis in which the patient is unable to abduct the affected eye to the midline. According to Lyle (1950) the operation, if adequately performed, should restore abduction to at least several degrees—and sometimes to as much as 25°—beyond the midline, and provide the patient with a good range of binocular single vision.

None of the other operations we have been considering has become really popular. For a full discussion of the indications for them, and the operative technique, the reader should consult Peter's (1936) monograph.

Muscle Transfer for Sphincter Lesions

History

Muscle transfer has been used in the treatment of urinary incontinence, especially stress incontinence in women, and also in the treatment of faecal incontinence.

The treatment of urinary stress incontinence by this means was initiated by Giordano (1907), who detached the gracilis muscle from its insertion and wrapped the muscle around the urethra. Goebell (1910) used the pyramidalis muscles, which he dissected free except at their insertion to the pubic crest, passed down on each side behind the pubis, and sutured together beneath the urethra. Subsequently this operation was modified by Frangenheim (1914), who included some of the rectus sheath with the pyramidales, and when these muscles were poorly developed used strips from the recti instead; by Thompson (1923), who turned down strips of rectus muscle and sheath in front of the pubes and sutured them together beneath the urethra; and by Miller (1932) who adopted a technique similar to that of Thompson except that he used strips fashioned from the pyramidales instead of from the recti. Squier (1911) transplanted strips of levator ani muscle between the urethra and the vagina, and Martius (1929) transplanted the bulbocavernosus muscle to the same site.

Deming (1926) used Giordano's technique in a patient with epispadias associated with urinary incontinence, a condition which Denis Browne (1951) has aptly described as epispadias with pubovesical cleft.

Muscle transfer for faecal incontinence

was initiated by Schoemacher (1909), who fashioned bilateral strips from the gluteus maximus muscles and sutured them together anterior to the rectum to act as an anal sphincter. A similar operation was performed later by Chittenden (1930) in a patient whose rectum had been excised five years previously for carcinoma. The procedure was modified by Bistrom (1911), who constructed gluteal muscle flaps which overlapped each other and the anus, split them, drew the bowel through the openings thus created, and sutured the mucosa to the skin.

Knapp (1939), in a young woman ren-

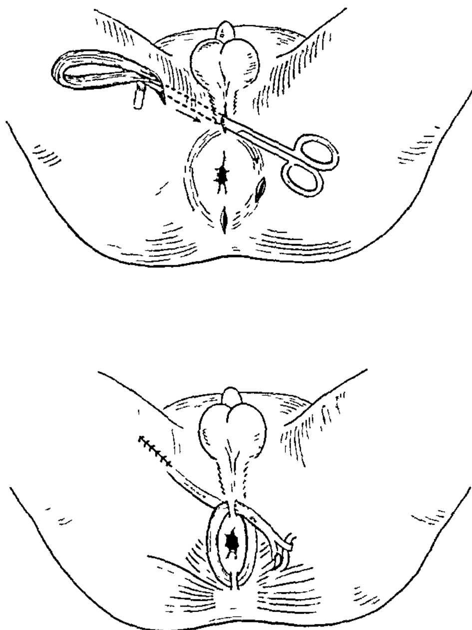


Fig 100 Construction of an anal sphincter by transfer of the gracilis muscle as described by Pickrell, Masters, Georgiade and Horton The muscle is detached from its insertion and its distal end is drawn upwards to the perineum It is made to encircle the anus lying subcutaneously except in the mid line where it is passed through openings in the anterior raphe and posterior raphe and is then anchored to the contralateral ischial tuberosity (Redrawn from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Dr Kenneth L. Pickrell)

dered incontinent by a previous operation for fistula-in-ano, freed the left superficial transverse perineal muscle from the ischial tuberosity to the central point of the perineum, leaving it attached at the central point, and used the muscle to fill the defect in the anal sphincter.

Pickrell and his colleagues (Pickrell, Broadbent, Masters and Metzger, 1952, Pickrell, Masters, Georgiade and Horton, 1954) developed a technique for constructing an anal sphincter by transferring the gracilis muscle (Fig. 100) in a manner reminiscent of Giordano's operation for urinary incontinence (*v supra*) The technique was designed primarily for the treatment of neurogenic anal incontinence due to spina bifida, and was used in 11 children suffering from this condition, in addition it was used in one child with a somewhat bizarre traumatic lesion* of the anal sphincter Pickrell *et al* have stressed the importance of preserving the branches from the profunda femoris artery and the obturator nerve which enter the upper part of the gracilis muscle They found that with proper operative technique and careful after-treatment the patients developed voluntary control of the new sphincter and became continent, but that full automatic control was not established.

*The child had been bitten by a sow'

Use in Present Day Surgery

Urinary stress incontinence in women which does not respond to conservative measures is now treated by the methods described in Chapter 15, and muscle transfer is rarely if ever used for the treatment of this condition or other forms of urinary incontinence.

In faecal incontinence, on the other hand, transfer of the gracilis muscle (Fig. 100) is being used increasingly with good results *The established indication* is neurogenic anal incontinence, but it seems likely that the operation will have a place as a secondary procedure in patients in whom a colostomy has had to be performed in infancy on account of rectal agenesis *

*This term, introduced by Denis Browne (*see* Browne, 1951), includes all congenital anomalies of the rectum and anus in which the anal sphincter has failed to develop and the gut either ends blindly or is connected to the urethra or vagina by a fistula. It does not include the conditions termed by Browne *microscopic anus* and *ectopic anus in which a sphincter is present with "normal nerve control for opening and closing"*

HETEROTOPIC TRANSPLANTATION OF MUSCLE

Interposition of Muscle in Arthroplasty

History

Verneuil (1860) interposed muscle between the bony surfaces in performing arthroplasty of the temporomandibular joint *During the next five or six decades* interposition of muscle, or muscle and fascia, was used by many surgeons as an alternative to interposition of fascia alone in performing arthroplasty of various joints, especially the elbow (for review *see* MacAusland, 1921).

Use in Present Day Surgery

The present status of arthroplasty, and the techniques used, have been discussed in Chapter 15. Operations in which muscle is interposed between the bones are now obsolete.

Free Transplantation of Muscle to Procure Haemostasis

History

It was found independently by Cushing (1911) in the United States and Horsley (1914) in Britain that bleeding from soft tissues, and in particular from the meninges, could often be controlled by applying a small piece of fresh autologous muscle to

the site of the bleeding.* Horsley experimented also with fascia and with boiled muscle but found that fresh muscle was much more effective. Subsequently Risley (1917) showed in dogs that haemorrhage from solid viscera could be controlled to some extent by autotransplants of fat, fascia or muscle, but confirmed that muscle transplants were the most effective.

Autologous muscle is not always readily available at the site of operation and to avoid making a separate incision some neuro-surgeons have used homologous or heterologous muscle (*see* Ingraham, Bailey and Nulsen, 1944).

Use in Present Day Surgery

The haemostatic effect of a muscle transplant depends, as Horsley (1914) pointed out, on the fact that it adheres readily to the site to which it is applied, and that it liberates substances which promote clotting. To obtain the greatest effect the muscle should be hammered into a thin sheet.

The technique is occasionally useful in various branches of surgery, but has been used mainly by neuro-surgeons to control oozing from large venous channels and from small vessels unsuited to ligation. It has the disadvantage however that even autologous muscle evokes a considerable reaction when transplanted to the brain (Ingraham, Bailey and Nulsen, 1944), and nowadays the same effect can be achieved more safely and certainly, and as a rule more conveniently, by using a prepared material such as fibrin foam or gelatin sponge, applying it dry or, if this is not effective, after soaking it in a solution of ox thrombin.

*According to Earl Walker (Walker, 1951, p. 61) Horsley probably used muscle before or at the same time as Cushing though his work was not reported until later.

CHAPTER 17

Transplantation of Nerve

Before discussing nerve transplantation it will be helpful to consider briefly the normal structure of the peripheral nerves and the changes which occur after injury, and their bearing on the treatment of nerve injuries.

STRUCTURE OF A PERIPHERAL NERVE

A peripheral nerve (Fig. 101) consists of numerous nerve fibres and supporting tissue arranged in one or more bundles commonly termed *funiculi*. The fibres are of two kinds, medullated and non-medullated, the former being much the more numerous. Each consists of a central core, the *axon*, which is the cytoplasmic process of a neuron, and a surrounding *axonal sheath*.

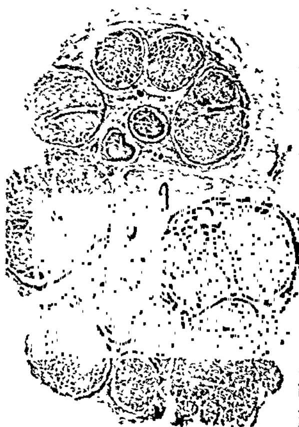


Fig. 101. Transverse section of the internal popliteal nerve. Haematoxylin and eosin. ×17.

The axon of a medullated fibre arises from a neuron situated either in the central nervous system or in a posterior root ganglion (or its cranial homologue). The axonal sheath consists of a layer of myelin, a layer of nucleated cells termed Schwann cells, a clear membrane termed the Schwann sheath or neurilemma,* and a layer of delicate connective tissue known as the endoneurium. At intervals of about a millimetre the continuity of the myelin sheath is interrupted and the Schwann sheath is turned inwards, giving the appearance of constriction. The sites at which this occurs are termed the nodes of Ranvier (Fig. 102).

The non-medullated fibres are either post-ganglionic efferent fibres of the sym-

*This is the sense in which the term neurilemma is used by Holmes and Young (1942) and Sunderland (1951). Some British anatomists, however, use the term to include also the Schwann cells. A good deal of confusion has arisen because Schwann himself, and also Cajal (1928), used the term neurilemma to denote the connective tissue constituting the perineurium and epineurium (p. 319)

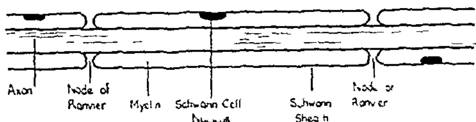


Fig 102 *Diagram representing a longitudinal section of a medullated nerve fibre and its coverings.*

pathetic system whose axons arise from neurons situated in the ganglia of the sympathetic trunk, or afferent fibres The axonal sheath of a non medullated fibre contains no myelin but is otherwise similar to the sheath of a medullated fibre †

Each nerve bundle is held together by a connective tissue sheath termed the *perineurium*, and the series of bundles which make up the nerve trunk are held together by a third system of connective tissue termed the *epineurium*

Most peripheral nerve trunks are composed of several funiculi, and these, as Sunderland (1952) has described, repeatedly divide and anastomose along the length of the nerve, in consequence there is a regrouping of the nerve fibres as they are traced proximally from the point where they leave the nerve as individual branches (Sunderland, 1953 Sunderland and Ray, 1948), and the size and number of the funiculi differ greatly at different levels In addition there is considerable variation in the proportion of the total cross sectional area occupied by epineural connective tissue at different levels

The nerve fibres seen in any given cross section vary considerably in diameter The range of variation is greatest in mixed nerves, efferent fibres and afferent proprioceptive fibres being as a general rule larger than afferent fibres subserving cutaneous sensibility (*see* e g Gutmann and Sanders, 1943)

Arterial branches reach a nerve at intervals along its course and form an anastomosing chain in the epineurium (*see* Adams, 1942, Durward, 1948, Causey, 1955) As a rule the anastomosis is so free that division of one or two of the entering vessels causes no damage to the nerve, local degeneration may result, however, if vessels in the epineurium itself are damaged or if many entering vessels are divided (Adams, 1943 Durward, 1948, Causey, 1955) Ischaemic damage in human nerves commonly occurs in association with Volkmann's contracture (Holmes, Highet and Seddon, 1944), and has been demonstrated in the peripheral stump of a divided nerve (Seddon and Holmes, 1944b)

† The foregoing descriptions are based on ordinary histological observations In recent years however the relationship of the Schwann cells to the axons has been studied by electron microscopy and it has been shown that axons of small calibre, both myelinated and non myelinated are enfolded within the Schwann cell cytoplasm and are connected to the cell surface by mesaxons (Gasser 1952 Causey and Hoffman, 1956)

NERVE INJURIES

CLASSIFICATION

Seddon (1943, 1950), in the light of extensive clinical experience, subdivided nerve injuries into three types injuries causing only transient nerve block, injuries in which axons are completely interrupted but the

Schwann tubes and endoneurium are preserved, and injuries in which the nerve is divided or otherwise completely disorganized. He adopted a suggestion of Sir Henry Cohen and termed these *neurapraxia*, *axonotmesis* and *neurotmesis* respectively.

This classification did much to dispel the confusion which had arisen regarding the results of peripheral nerve injuries, but it has been criticised on two grounds. In the first place many have objected to Seddon's rather cumbersome Greek terms. Secondly, and of greater significance, there are injuries which are more extensive than axonotmesis but which fall short of neurotmesis, using these terms strictly as Seddon defined them.

Another classification has therefore been proposed by Sunderland (1951), who recognizes five degrees of injury of ascending grades of severity. In injuries of the first degree conduction is blocked at the site of injury but the continuity of all components comprising the nerve trunk, including the axons, is preserved. In second degree injuries there is disintegration of axons but the continuity of the axonal sheaths and other structures comprising the nerve is preserved. In third degree injuries the internal structure of the funiculi is disorganized though the bundles remain in continuity and show little, if any, deformation of their outlines. In fourth degree injuries the funiculi are breached and disorganized so that they are no longer sharply demarcated from the epineurium in which they are embedded, but continuity of the nerve trunk is preserved. Fifth degree injuries are those in which the nerve trunk is severed.

Various partial and mixed lesions also occur. Thus, for example, some of the funiculi may be completely severed while others escape, and in a given funiculus different fibres may show different degrees of injury.

EFFECTS OF INJURY.* DEGENERATION AND REGENERATION

After first degree injuries the condition is completely reversible and perfect functional recovery occurs. Opportunities for histological observations on human nerves are limited but it is known that conduction may be blocked without detectable histological changes in the nerve (*see* Sunderland, 1951).

After other grades of injury the affected fibres undergo a process known as Wallerian degeneration distal to the point of injury and, in the case of medullated fibres, proximally up to the nearest node of Ranvier. The axons and their myelin sheaths break up and disappear in the course of a few weeks, due partly to the activity of macrophages which accumulate in large numbers about 8 days after injury and gradually disappear after about the 25th day (Holmes and Young, 1942), and the Schwann cells enlarge and proliferate; in consequence, at the site of each axon, a tube, walled by endoneurium and containing Schwann cells and phagocytes, is formed. Subsequently shrinkage occurs so that each of the "Schwann tubes," as they are called, becomes smaller in diameter than the axon whose disappearance led to its formation. While this process is going on axons grow out from the injured fibres proximal to the level of injury and enter the Schwann tubes.

In second degree injuries each regenerating axon enters the Schwann tube formed by degeneration of its distal portion and eventually makes connexion with the same end organ as before, so that complete recovery occurs.

In third degree injuries the regenerating axons, though still confined within the funiculus, are no longer confined to the endoneural tubes which originally contained them. In consequence some enter foreign tubes while others are blocked as a result of intrafunicular fibrosis. The chances of er-

*The clinical features of nerve injuries will not be considered. They are fully described in many papers, monographs and textbooks (*see* e.g. Sunderland, 1951; Medical Research Council, 1954).

-oneous cross shunting of axons is increased by axonal sprouting which occurs during regeneration (Sunderland 1951 Shawe 1955) and results in an excess of axons over endoneural tubes The functional recovery may be still further impaired by retrograde degeneration of some of the neurons whose axons are divided (Sunderland 1951 1952)

In fourth degree injuries regenerating axons are left free to enter the interfunicular spaces and many become lost and terminate *blindly* while *others* enter *foreign* tubes The axonal loss and erroneous cross shunting is increased by axonal sprouting (*v supra*) and by the severe *fibrous tissue* reaction which occurs and the functional recovery is still further impaired by retrograde neuronal degeneration which occurs to a greater extent than with third degree injuries

The histological findings after fifth degree injuries depend on the extent of separation of the nerve ends as well as on the time which has elapsed since the injury

If the nerve ends are widely separated the regenerating axons may *fail to* reach the distal segment and bulbous enlargements known as *traumatic neuromata,* consisting of scar tissue and nerve fibres are formed If however the nerve ends are brought into apposition or nearly so before scar tissue has formed a considerable number of axons enter the Schwann tubes and some of them under the influence of the Schwann cells acquire myelin sheaths and continue to grow distally until they establish connexion with end organs Despite this functional recovery may be very imperfect on account of the high incidence of retrograde neuronal *degeneration* axonal *loss* and erroneous cross shunting (Sunderland 1951 1952)

It should be observed that even in a purely sensory nerve erroneous cross shunting of axons is *harmful* because a neuron normally subserving one type of sensation which becomes connected with an end organ concerned with some other type of sensation is unlikely to function efficiently it is even more disastrous however, in a nerve carrying both afferent and efferent fibres *

Many attempts have been made to determine the rate of regeneration both in experimental animals and in man Young and Medawar (1940) *found* after dividing and suturing the sciatic nerve in rabbits that the axon tips advanced at an approximately constant rate of 3·9 mm day Later work has shown however that over long distances *the rate progressively decreases* In *man* various figures have been given for the rate of advance of axon tips ranging from 1·1 to 2·6 mm /day depending on the particular nerve and the grade of injury (for review *see* Bowden and Sholl 1951) The rate of regeneration as judged by functional recovery is slower but so variable that, according to Bowden and Sholl (1951), it is not possible at present to find a useful and reliable figure for the average rate of recovery '

PRINCIPLES OF TREATMENT

In all cases areas rendered anaesthetic must be protected from injury and over

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* There are divergent views concerning the extent to which the diameter of the Schwann tubes in the distal stump limits the diameter of the regenerating fibres Sanders and Young (1944) found in rabbits that the Schwann tubes preserved the pattern of fibre size in the peripheral stump during degeneration and controlled the *pattern of fibre size appearing as the result of re*generation by restricting the diameter of the entering fibres. On the other hand a year later Simpson and Young (1945) stated that regenerating large diameter fibres can enter and inflate Schwann tubes formed by the degeneration of small fibres.

Sunderland (1952) has pointed out that after division of human nerves gross shrinkage occurs in the Schwann tubes of the distal stump and that three months after injury the tube diameter is rarely greater than 3 microns Despite this "the distal stump retains the capacity for at least twelve months of transmitting axons to *the* periphery in a manner that does not differ significantly from that observed when repair is undertaken immediately and the muscle function can be fully restored following reinnervation when the distal stump has been denervated for the same period " He suggests therefore that in man either the Schwann tube shrinkage is reversible or alternatively it may not be necessary for the recovery of muscle function that the nerve fibres should be restored to their original diameter "

stretching of paralysed muscles must be prevented.

In injuries of the first, second or third degree no other treatment is indicated, but in fourth degree injuries it is usually advantageous to excise the damaged segment and perform end-to-end union. The clinical indications for operation in such cases have been discussed in detail by Zachary and Roaf (1954).

When a nerve has been completely divided the further treatment depends on the state of the wound.

If there is virtually no contamination, and the nerve has been cleanly divided without being crushed, immediate repair is indicated As Blackwood and Holmes (1954) have pointed out, however, such ideal conditions, though easily attainable in the laboratory, are rarely present in clinical practice, and better results are often obtained if repair is postponed for a few weeks even when contamination appears minimal. The main reason for this appears to be that if immediate repair is undertaken intraneural damage on either side of the line of section, due to direct injury or ischaemia, may pass unrecognized at the time and give rise to serious fibrosis later

When the wound is seriously contaminated repair of the nerve should not be attempted until infection has been controlled and the wound has healed

End-to-End Union

Methods of Obtaining Apposition

After division of a nerve end-to-end union is indicated, and can usually be performed without difficulty, when there is no loss of substance and repair is undertaken soon after injury When part of a nerve has been destroyed, or repair has been delayed and scarring has occurred necessitating excision of the nerve ends, the problem is more difficult. It may still be possible to bring the ends into apposition by mobilizing the nerve above and below the level of injury, by transposing or stretching the nerve, or by shortening the limb, but the advantages and disadvantages of these manoeuvres must be considered before deciding whether to proceed with end-to-end union or to adopt one of the alternative procedures such as nerve grafting.

Mobilization is a widely used and valuable procedure. Small branches may be stripped up and in some cases sacrificed provided that the longitudinal vascular chain is carefully preserved.

Transposition is also very useful but is of more limited application. The most important examples are transposition of the ulnar nerve at the elbow from the back to the front of the medial epicondyle of the humerus, and transposition of the radial nerve in the arm from the back to the front of the humerus.

Nerve stretching is a more questionable procedure Sudden stretching at the time of operation is futile,* but some good results have been obtained by gradual stretching One method is to bring the nerve ends together after excising any scar tissue by flexing appropriate joints, suture them with the limb in this position, and subsequently stretch the nerve by gradually bringing the limb back to a normal position over a period of weeks or months. A modification termed bulb suture, which was suggested by Naffziger (1921) and is applicable when repair is delayed, is to suture the nerve without resecting the traumatic neuromata, and excise

*The effect of sudden stretching was studied in cats by Denny-Brown and Doherty (1945). They found that mild stretching caused damage to epineural vessels with resultant patchy ischaemic changes. More severe stretching caused rupture of the perineurium and herniation of the nerve bundles. Following slight herniation a localized swelling formed but this was due mainly to oedema and did not prevent subsequent regeneration After gross herniation endoneural fibrosis occurred and regeneration was seriously impaired

These investigators found also that a nerve of small calibre was much more tolerant of stretching than one of large calibre.

these later at a second operation after the nerve has been stretched

Nerve stretching was widely used during and for some years after the first world war (Stopford 1920 Medical Research Council 1920) but subsequently waned in popularity because it was realized that the nerve might be severely injured even with gradual stretching (*see* Highet and Sanders 1943 Highet and Holmes 1943)

More recently Seddon (1954) after reviewing 44 cases has concluded that bulb suture has a definite place in the repair of large gaps in main nerves except when a second nerve in the limb has been damaged beyond hope of repair and can be used as a graft Seddon has advised that in performing bulb suture the elbow or knee should not be flexed beyond 90° and the rate of extension after operation should be controlled by a turnbuckle incorporated in the plaster and should *not* exceed 3° *per day* Under these conditions it has been possible to perform suture when the gap produced by the injury and by the necessary resection of the stump neuromata was as much as 11 cm and in one case reported by Seddon a gap of 13 5 cm in the median nerve was successfully bridged by first performing bulb suture and subsequently inserting a 6 5 cm autograft

The problem has been further investigated experimentally in dogs by Hoen and Brackett (1956) Their results with gradual stretching were encouraging

Limb shortening by resection of bone is a formidable and distinctly hazardous procedure (*see* e g Swan 1944) According to Seddon (1954) it is no longer justifiable to shorten an intact bone except when this is the only means of closing large gaps in more than one nerve when an ununited fracture is present however the indications are somewhat less restricted

Technique of End-to-End Union

The usual procedure is to bring the nerve ends together orientating them correctly as far as can be judged with the naked eye and suture the epineurium only (Fig 103) Sometimes a suture passed through the whole thickness of the nerve is used in addition but this results in more scarring and is unnecessary if the ends can be apposed without tension Various suture materials have been tried including catgut silk hair tantalum stainless steel and nylon Catgut was reported by Sargent and Greenfield (1919) to produce more reaction than silk or silkworm gut and has since been avoided by most surgeons but according to Sunderland and Smith (1950) is probably the most satisfactory material provided that its sterility is assured and despite claims to the contrary causes minimal scarring Silk and human hair are also satisfactory (Guttmann 1943 Sunderland and Smith 1950) and the former is widely used Tantalum wire

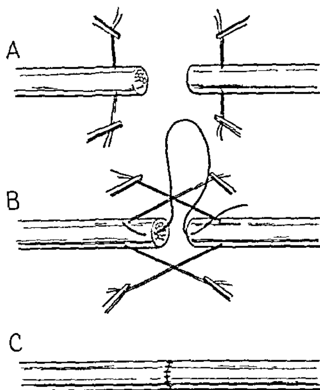


Fig 103 Nerve suture A The nerve ends have been prepared and are held correctly orientated by stay sutures B C Union is effected by interrupted sutures through the nerve sheath Occasionally one suture is passed through the whole thickness of the nerve in addition

strongly advocated by Spurling (1943), causes little reaction for weeks or months but, as Sunderland and Smith (1950) have shown, eventually causes more scarring than either silk or catgut, and the same appears to be true of stainless steel wire. Nylon [illegible] scarring and should not be used.

[illegible] *native method is to "glue" the* ends together with fibrin or some [illegible]esive substance. This procedure [illegible]duced, and studied experimentally [illegible] and dogs, by Young and Medawar [illegible] These investigators used heterolo[illegible] chicken plasma, and to obtain a suffi[illegible] firm clot they fortified it by adding [illegible] prepared from chicken plasma [illegible]ipitation with acetic acid. Imme[illegible] before use they mixed the fortified [illegible] with chick embryo extract, and they [illegible] the mixture with a pipette while [illegible] the nerve ends in apposition with [illegible] Young and Medawar claimed [illegible] fibres grew slightly faster across [illegible] made in this way than across junc[illegible] by conventional suture methods. [illegible] figures are not very convincing. [illegible] does have the advantage, how[illegible] minimal distortion of the [illegible] greatly facilitates the repair [illegible] calibre and the *insertion* [illegible].

[illegible] was first used clinically [illegible] Medawar (1942), who re[illegible]ded median nerve by the [illegible]escribed above. The technique [illegible] modified by Tarlov and his col[illegible] (Tarlov and Benjamin, 1942, 1943; Tarlov, Goldfar[illegible] Benjamin, 1942; Shapiro, Tarlov, [illegible] Goldfarb, Bojar, Kaslow [illegible], 1944; Tarlov, Denslow [illegible] and Pineles, 1943; Tarlov, 1944a [illegible] and Boernstein, 1948), who found experimentally that heterologous plasma caused a considerable inflammatory reaction, and that this could be avoided by using autologous plasma, either prepared without anticoagulants, or heparinized and subsequently made to clot by adding protamine sulphate (Shapiro *et al.*, 1944). Tarlov and his colleagues also designed useful rubber moulds by means of which the clot can be distributed around the whole circumference of the nerve. To facilitate accurate apposition and to give greater strength to *the junction they have advised inserting* a single tension stitch of tantalum wire (Tarlov, 1944a); for the reason discussed above, however, this choice of material may prove to have been unfortunate. The modified technique has been used clinically by Tarlov (1944b) with encouraging results. Seddon (1954) has used prepared human fibrinogen* and human thrombin,* which form a firm clot a few seconds after mixing, mainly for anchoring small calibre nerve grafts.

Another modification was introduced and used experimentally by Singer (1945), who used fibrin film, impregnated with thrombin and surfaced with fibrinogen, as a tubular splice. Singer found that the reaction was more marked than that evoked by conventional suture with hair or tantalum wire, but this may have been because he used heterologous fibrin.

Klemme, Woolsey and DeRezende (1943), *instead of using fibrin, glued the nerve ends* together with 50 per cent gum acacia. They reinforced the junction with flaps of nerve sheath and "allantoid membrane."

Yet another form of nerve splice was devised by Weiss (1943a, c, 1944), who joined nerves in experimental animals by means of sleeves consisting of freeze-dried arterial segments. He found it advantageous to use a sleeve having an internal diameter slightly less than the diameter of the nerve to be joined, and to stretch it temporarily with a special instrument (Fig. 104) to permit introduction of the nerve ends.

No final verdict on the value of glueing and sleeve splicing techniques can yet be

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*Obtainable in Great Britain from The Lister Institute, London.

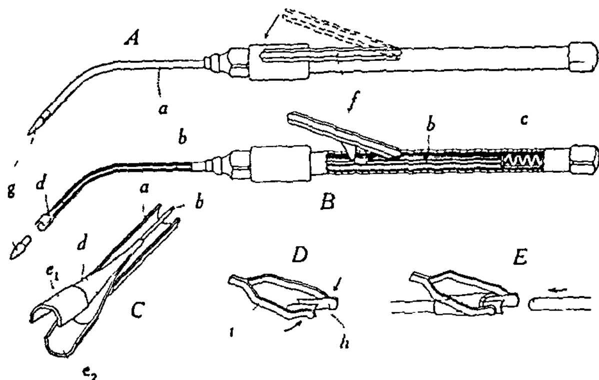


Fig. 101. Weiss instruments for splicing a nerve with a sleeve of freeze-dried artery. The grasping instrument illustrated in figures A, B, C *consists of a seamless steel tube (a) containing a movable flexible* wire (b). One end of (b) rests against a coil spring (c), the other is attached to a chuck (d) which ends in two half-cylinders (e_1, e_2). When the lever (f) is depressed the chuck is retracted and the jaws are closed; when this lever is elevated the chuck is forced outwards by the spring (c) and the jaws spring apart. In splicing, the lever is first depressed, the open end of the instrument is plugged by a cone (g) and the arterial sleeve is passed over the end of the instrument and on to the shaft (a). The lever is then raised, cone (g) is removed, one free nerve end is grasped between the jaws (e_1, e_2) by depressing the *lever again and the arterial sleeve is slipped on to the nerve. The open end of the sleeve is then* stretched by means of the spreader illustrated in figure D, the other nerve end is introduced as shown in E, after which the spreader is withdrawn. (Reproduced from *Proceedings of the Society for Experimental Biology and Medicine* by courtesy of the publishers and Dr. Paul Weiss.)

given, but it is clear that, while they may cause less distortion than conventional methods of suture, they still permit a considerable amount of erroneous cross-shunting of fibres which, as we have seen, is the main cause of the unpredictable and often disappointing results in this field of surgery.

A more fundamental attack on the problem was suggested as long ago as 1917 by Langley and Hashimoto, who argued that better results might be obtained by funicular suture, that is to say by isolating one or more bundles representing a branch or major division of a nerve and suturing them separately.

This suggestion attracted little attention for many years but recently Sunderland (1953) has re-opened the question in the light of detailed studies of the internal anatomy of the main peripheral nerves and has suggested several sites—notably the radial nerve in the spiral groove and at the level of the elbow, the ulnar nerve in the middle third of the forearm and at the wrist, the sciatic nerve, the proximal part of the lateral popliteal nerve and the distal part of the medial popliteal nerve—in which it should be advantageous to perform funicular suture or alternatively to exclude from the suture line bundles whose fibres are relatively unimportant and would, during recovery, complicate the regeneration of more important axons. As Sunderland has pointed out,

these operations must be performed under low power magnification, and they require a detailed knowledge of the internal anatomy of the injured nerve, but, as he says, these "should not be excessive demands to the enterprising surgeon."

Alternatives to End-to-End Union

When it has proved impossible to unite the ends of a divided nerve the following procedures have been tried:

1 Bridging the gap by (a) a free nerve graft, (b) a pedicled nerve graft, (c) a graft of non-nervous tissue, and (d) an implant of foreign material.

2 Joining the distal stump of the divided nerve to some other nerve. This procedure is termed nerve crossing

3 Muscle transfer (p. 308) or tendon transfer (p 303) to compensate for paralysis.

Nerve grafting and nerve crossing will be considered in detail.

Bridging by non-nervous tissue and implants requires only brief mention Assaky (1886) used strands of catgut with the object of providing a scaffolding to support and guide the regenerating nerve fibres, and during the next forty years an astonishing variety of materials were tried including strands of silk; tubes of decalcified bone, casein, rubber, magnesium, gelatin and fascia; and blood vessel segments, both fresh and formolized. This work has been reviewed by Sanders (1942), who concluded that all these procedures were of little value and certainly inferior to methods that provide "a milieu of live Schwann cells for the out-growth of nerve fibres." This conclusion is probably still valid, but it must be recorded that Weiss and his colleagues (Weiss, 1943a, c, 1944, 1945; Weiss and Taylor, 1946, Matson, Alexander and Weiss, 1948) have re-opened the question, and have reported some success with bridges in the form of blood vessel segments* containing either blood clot alone, or strands of nylon or tantalum. In addition Causey (1955) has shown in rabbits that nerve fibres may traverse a pedicled muscle graft.

*Fresh or freeze-dried.

FREE NERVE GRAFTING

HISTORY

In reviewing the history of free nerve grafting† it will be convenient to consider separately the use of fresh and predegenerated autografts, fresh and stored homografts, and fresh and stored heterografts, in animal experiments and clinical practice.

Fresh Autografts

Animal Experiments

Phillipeaux and Vulpian (1870) showed that a free autograft used to fill a defect in another nerve became firmly united to the host nerve and after a time was capable of conducting an impulse. They used a graft from the lingual nerve to the hypoglossal nerve in a dog, and after 71 days demonstrated contraction of the muscles of the tongue when the host nerve was stimulated electrically proximal to the graft. Similar observations with grafts in other nerves, in various species including dogs, rabbits, cats and monkeys, were made subsequently by Kilvington (1908), Ingebrigsten (1916), Duel (1933), Bentley and Hill (1936), Gutmann and Sanders (1942), and others. Kilvington's paper, which is extraordinarily informative considering when it was written, records that good results were obtained with autografts from various sites, even afferent nerve segments functioning satisfac-

†This section is based largely on the excellent review by Sanders (1942), and for later work on the Medical Research Council (1954) Special Report (No 282).

tonly when transplanted to mixed nerves and that it made no difference which way the graft was orientated

Histological studies have shown that nerve autografts undergo Wallerian degeneration with proliferation of Schwann cells (Merzbacher 1905 Ingebrigsten 1915 Sanders and Young 1942 Sanders 1949) and are penetrated by regenerating fibres from the host nerve in the course of a few weeks (von Bunger 1891 von Notthafft 1893 Bielschowsky and Unger 1917 Huber 1920 Bunnell and Boyes 1939 Young Holmes and Sanders 1940 Sanders and Young 1942 Gutmann and Sanders 1942 Sanders 1949) With thick grafts as Maccabruni (1911) and Bielschowsky and Unger (1917) pointed out there may be a zone of central avascular necrosis in which regeneration either fails to occur or is long delayed whereas with thin grafts avascular necrosis is rare (Maccabruni 1911 Bentley and Hill 1936 Sanders and Young 1942) in consequence Huber (1920) and many later investigators have advocated the use of cable grafts consisting of several strands for bridging defects in nerves of large calibre

Davis and Cleveland (1934) suggested that scar tissue might form at the line of union between the graft and the distal segment of the host nerve in sufficient amount to act as an impenetrable barrier to the regenerating axons and therefore advised resecting and resuturing this junction when sufficient time had elapsed for axons to reach it Gutmann and Sanders (1942) have opposed this however on the grounds that scarring can be minimized by proper technique and that when it does occur it is by no means confined to the distal junction Fears that

ing defects in motor nerves were expressed by Gutmann and Sanders (1943) these have been partly allayed by the observation of Simpson and Young (1945) that large medullated fibres can inflate Schwann tubes formed by degeneration of smaller fibres but further study is required to determine the extent to which this process can occur

Return of voluntary movement and reflex action after nerve autografting has been studied by Ballance and Duel (1932) who found partial recovery from facial paralysis in baboons monkeys and cats 128 to 166 days after inserting a graft in the facial nerve and Gutmann and Sanders (1942) who found recovery of the spreading reflex in rabbits 51 to 98 days after inserting a 2 cm autograft in the peroneal nerve

Recovery of cutaneous sensation after grafting has not been studied extensively in animals Huber (1919) reported evidence of sensory regeneration but gave no details of his observations Gutmann and Sanders (1942) demonstrated complete sensory recovery in one rabbit 171 days after inserting a 2 cm graft into the peroneal nerve in three other rabbits similarly treated however there was no recovery of sensation within the autonomous zone of the nerve after 162 182 and 200 days respectively

Clinical Observations

Many of the earlier cases of nerve autografting are of little or no help in assessing the value of this procedure some because they were not followed for a sufficient time or reported in enough detail others like the cases reported on so pessimistically by Platt (1919 1921) and Stopford (1920) in which the grafts were of smaller calibre than the host nerves and were surrounded by fascial sheaths containing olive oil or petroleum jelly because the operations were in the light of present knowledge technically unsatisfactory

Sanders (1942) after eliminating cases judged unsatisfactory for one or other of these reasons found 101 cases in which fresh autografts were used to repair defects in limb nerves up to the end of 1939 The first of these was a patient operated on by Dean in 1896 and cited by Sherren (1906), in

whom a 3 inch gap in the musculospiral nerve was repaired—apparently with excellent result—by a graft from the superficial radial nerve. Of the other 100 cases Sanders found that 81 showed some evidence of improvement.* In 50 of the cases—reported mainly by Swan (1919), Joyce (1919, 1920), Stookey (1922), Bunnell (1927), Foerster ([illegible]) and Bunnell and Boyes (1939)—there were sufficient data to admit of a more accurate assessment, and of these Sanders classed 25 as markedly improved, 21 as slightly improved and 4 as not improved. The great majority of the grafts in these patients were either cable grafts or thin grafts in digital nerves,† and this fact may possibly account for the surprisingly good results.

Further reason for questioning the soundness of the extremely pessimistic attitude towards nerve grafting which developed among British surgeons towards the end of the first world war (see e.g. Souttar and Twining, 1918; Platt, 1919, 1921; Sargent, 1920; Stopford, 1920; Medical Research Council, 1[illegible]; Platt and Bristow, 1924), and has persisted in some quarters ever since, was provided by observations on the results of free grafts in the treatment of injuries of the facial nerve.‡ This method of treatment was suggested by Bunnell (1927) for use in cases where direct suture of the nerve was impossible, and was successfully used by him in 1930 to repair a 6.3 cm. defect in the facial nerve resulting from resection of a tumour. Bunnell's cases were not reported at the time,* and in the same year Ballance and Duel began using free grafts experimentally to repair defects in the facial nerve in baboons. In 1931 Duel (reported by Ballance and Duel, 1932) inserted a 2.7 cm. graft in an infant of 8 months whose left facial nerve had been injured during a mastoid operation two days previously and obtained an excellent result, and subsequently he and Ballance published a series of papers reporting their experimental work and numerous clinical cases (Ballance and Duel, 1932; Duel, 1932, 1933, 1934; Ballance, 1934; Duel and Tickle, 1936). They took the grafts from various sources including the long thoracic nerve, the anterior cutaneous nerve of the thigh, the sural nerve, the intercostal nerves and the intercosto-brachial nerve, and concluded that "any nerve, sensory or motor, will function equally well for the purpose, as long as it is of suitable size to be in the fallopian canal." The operations were often performed in the presence of pus, and as a rule the grafts were not sutured but were covered temporarily with gold foil and the wounds were packed. Response to faradic stimulation returned some weeks after grafting for recent injuries and some months after grafting for long standing injuries, and always preceded return of spontaneous movement. Though detailed follow-up reports are often lacking it is clear that many good functional results were obtained, and it seems likely that the opera-

* The largest series included here is that of Gosset and Charrier (1922), who reported improvement in 16 out of 30 cases. Their attitude reflected the prevailing opinion of surgeons in both France and Germany (see e.g. Lexer, 1909; Foerster, 1916; Depage, 1918; Gosset, 1918), but unfortunately their cases were reported so briefly that it is impossible to assess them properly.

† Sanders says all were of these types, but this is not quite correct. Joyce (1919), for example, in four patients used a single strand from the superficial radial nerve to bridge a gap in a nerve of larger calibre. It may be remarked in addition that he wrapped the grafts in Cargile membrane, and his cases are therefore to some extent open to the same criticisms as those reported by Platt (q.v. supra).

‡ Up to 19[illegible] all attempts to repair the facial nerve were by nerve crossing and, as we shall see, this operation had a considerable vogue for many years though it is rarely performed now. Sydenham ([illegible]) and Marsh ([illegible]) used bridges of silkworm gut and catgut respectively and claimed good results, but their methods have long since been abandoned. Direct suture, used by Ney (1922), Bunnell (1927, 1937) and Martin (1931), is still regarded as the ideal method when feasible, but the gap is sometimes so large that the stumps cannot be brought together even if the nerve is re-routed. To deal with such cases two fundamentally different procedures were evolved: free nerve grafting, and correction of the deformity by the insertion of fascial slings or by muscle transfer.

* The first full account appears to be that published in 1937.

tions would have been even more successful but for the presence of infection. In the great majority of these cases grafting was undertaken for repair of the nerve following injury within the facial canal during mastoid operations, and this has continued to be the main indication for the procedure (*see* e.g. Foster, 1935, Martin, 1936, Morris 1939). Fresh autografts have also been used with some success, however, for the treatment of facial nerve injuries resulting from the removal of tumours of the parotid gland (Conley, 1955).

Interest in the use of nerve grafts to repair large defects in limb nerves increased greatly during and shortly after the second world war.

Klar (1943) reported 21 cases treated in this way, 20 of whom received cable grafts. Five patients showed recovery of some function in all the affected muscles, and 5 others showed some recovery in some muscles.

Seddon (1947) criticised Klar's technique on the ground that the total cross sectional area of the graft was usually less than that of the host nerve, and also his method of assessment. In the same paper Seddon reported 58 cases himself and subsequently (Seddon, 1950, 1954) increased the number to 73. In assessing these cases he described the operation as successful if, and only if, the result was as good as the best observed after secondary suture of the same nerve for repair of a short gap at the same level as in the grafted case. Out of 21 digital nerve grafts in 16 patients 11 were successful by this criterion, and 2 gave evidence of partial recovery. Cable grafts were successful in 7 cases out of 17 and there was evidence of partial recovery in 6 others. Main trunk grafts to the median nerve were successful in 9 cases out of 15, and in 4 other cases there was some evidence of recovery; similar grafts to other nerves were successful in 5 cases out of 19, and in 5 other cases there was some evidence of recovery. Inlay grafts in partly divided nerves were successful in 4 cases out of 6, and 1 of the other 2 cases was reported as recovering.

Seddon, Young and Holmes (1942) made interesting histological observations on a cable graft which had been used unsuccessfully to bridge a gap in the median nerve. They found seven months after grafting that nerve fibres had entered the graft and had acquired myelin sheaths and they attributed the poor result to inadequate resection of scar tissue at the original operation. Histological observations in two other cases of Seddon's were reported subsequently by Holmes (1947).

Titrud (1947) reported a single case in which the musculospiral nerve was successfully repaired with a cable graft. Bjorkesten (1948) used cable grafts in 4 cases, with some recovery in 2. He also used fresh main trunk grafts to repair defects in the median and sciatic nerves. Of the 7 grafts to the median 2 yielded good results and 3 others some improvement, the 2 grafts to the sciatic, however, yielded 1 poor result and 1 failure.

Predegenerated Autografts

Animal Experiments

Tello (1911) observed that pieces of fresh nerve transplanted to the brain were absorbed whereas pieces of degenerated nerve attracted brain fibres, in consequence he suggested that predegenerated autografts transplanted orthotopically might be penetrated by new fibres more rapidly than fresh grafts. Huber (1920) investigated the matter experimentally without finding any evidence to support Tello's prediction, but the possible advantages of predegenerated grafts were again urged by Cajal (1928). Further trial by Duel (1933), who used grafts of both types to repair defects in the facial nerve in monkeys, suggested that regeneration did occur more rapidly with predegenerated grafts, but subsequent observations on the repair of other nerves in cats (Bentley and Hill, 1936, Bunnell and Boyes, 1939) and

nerve, two showed complete sensory recovery after 172 and 185 days respectively, one showed partial recovery after 165 days, and the remainder showed no signs of recovery after 165-200 days.

In the light of the above findings, and of clinical observations to be considered later, it was suggested by Seddon and Holmes (1944a), and by Barnes, Bacsich, Wyburn and Kerr (1946), that the histological changes in nerve homografts could be accounted for by postulating that such grafts, like homografts of skin and other tissues, evoke a state of immunity in the host, in consequence of which they are eventually destroyed, but that re-innervation proceeds until immunity is established. If, as now appears virtually certain, this explanation is correct, it would seem to follow that, other things being equal, the longer the gap to be bridged the less the chance of a homograft being successful and, secondly, that the maximum length of gap capable of being bridged successfully by a homograft would be decreased by procedures which hastened the development of immunity in the host and increased by procedures which either delayed the development of immunity or protected the graft—even temporarily—from the consequences of such immunity.

Sanders (1954a, b) argued that a long graft was likely to be disadvantageous not only because of the length of the gap to be bridged by host fibres but also because of the correspondingly high dosage of foreign tissue which, by analogy with skin grafts (p. 45), might be expected to hasten the development of immunity. In experiments designed to test this prediction in rabbits he found that host fibres did not extend as far in 5 cm. homografts examined after 30 days as they did in 2 cm. grafts examined after 25 days; moreover after inserting 5 cm. grafts there was never any return of cutaneous sensibility, and motor recovery occurred more slowly and less completely than with 2 cm. grafts.

Sanders' postulate of a dosage phenomenon in nerve homografts has not passed unchallenged (Rogers, 1954b) and, as he himself has admitted, "the data on dosage are extremely scanty." Another approach to the problem is to try to modify either the graft or the defence mechanism of the host in such a way that the development of immunity to grafts of constant size is delayed. Sanders (1954a, b) subjected nerve homografts to repeated freezing and thawing with this object in view and found that after 30 days host fibres had penetrated significantly further into such grafts than into fresh homografts.

Some other observations of significance in this connexion will be considered later when we discuss the behaviour of stored homografts. There appear to have been no serious attempts, however, to modify the defence mechanism of the host to nerve homografts, and it would seem worthwhile to study the effect of the various procedures discussed in Chapter 6 which have been shown to delay the development of immunity to homografts of skin and other tissues.

Clinical Observations

Albert (1885) appears to have been the first to use a nerve homograft in man. He obtained the graft from an amputated limb. No improvement was observed but the patient was followed far too short a time for this to be of any significance. A few years later Mayo Robson (1889) published a preliminary report of a case in which a 2½ inch defect in the median nerve resulting from resection of a tumour was successfully bridged by a homograft, and subsequently (Robson, 1917) he stated that three years after grafting "recovery was perfect in every respect." It is difficult, however, to assess the reliability of these reports in view of the statement by Atkinson (1890) that sensation had returned to a considerable extent in this patient 36 hours after operation and was normal in five weeks.

Sanders (1942) found in the literature records of 12 cases treated by homografts 10 of which had been reported by Dujarier and François (1918) According to Sanders there was evidence of some degree of recovery in 8 of these as Seddon and Holmes (1944a) have pointed out however careful scrutiny of the data fails to reveal any convincing evidence of recovery except in 3 of the 6 cases reported by Duel (1934) in which homografts were used to treat injuries of the facial nerve

More recent observations have reinforced the attitude of pessimism evoked by the earlier reports

Seddon and Holmes (1944a) Spurling Lyons Whitcomb and Woodhall (1945) and Barnes Bacsich Wyburn and Kerr (1946) reported between them 18 cases in which fresh homografts were used and in none of them was there any recovery of function These grafts were explored and biopsied after they had been *in situ* for periods ranging from 110 to 901 days It was found that the grafts had united to the host nerve though there was often quite a large stump neuroma at the proximal junction Some of the grafts had shrunk considerably in diameter others had retained their original calibre but over most of their length were pale in colour abnormal in consistence (some being almost as firm as tendon others quite soft) and often densely adherent to surrounding tissue Histological examination showed that the proximal 10 15 mm was often re innervated by regenerating host axons though it differed from an autograft in containing numerous collections of small round cells More distally necrotic material containing numerous macrophages and scar tissue were found in varying proportions with no regenerating fibres

Some years later Davis and Ruge (1950) reported a further series of eight cases in which homografts were used and again functional recovery was either absent or minimal

In spite of these discouraging results it would in the author s *opinion* be premature to conclude that nerve homografts will never be of any clinical value unless and until some method is discovered by which homografts in general can be made to survive indefinitely It seems clear from the preceding discussion that nerve grafts fall in the *category of static grafts* (p 211) and that for them to become useful it would suffice to discover means of prolonging their survival sufficiently for re innervation to be completed It may well be that this more limited objective will be attained before a general solution of the homograft problem is discovered

Stored Homografts

Animal Experiments

The following methods of storage have been tried

1 Storage at 37 C in 0 9 per cent NaCl
2 Storage at ice box temperatures on ice or in petroleum jelly liquid petrolatum 0 9 per cent NaCl or Ringer s solution
3 Storage in chemical preservatives
4 Storage at room temperature after *freeze drying*

Tello (1914) found that nerve segments stored in 0 9 per cent NaCl at 37 C for three weeks remained viable and were re inner vated when used as homografts

Bethe (1916) observed re innervation of nerve homografts in dogs after storage on ice for a few days Huber (1920) following a suggestion of Dujarier and François (1918) stored homografts of rabbit nerve in petroleum jelly or liquid petrolatum at 2 C for several weeks and found that they became re innervated as quickly as fresh homografts and gave excellent functional results Eden (1919) had little success with a dog homograft stored at ice box *temperature in 0 9* per cent NaCl for only three days but more recently Sanders and his colleagues (Sanders and Young 1942 Gutmann and Sanders

1942) have obtained encouraging results with rabbit homografts 2 cm. long stored at 2 C. in Ringer's solution for one to three weeks and then inserted into defects in the peroneal nerve

In the experiments of Sanders and Young the grafts were removed for histological examination after 25 days in the host They were rarely so swollen as fresh homografts, and adhesions between graft and bed were slight or absent Grafts stored for one week were better vascularized than those stored for two or three weeks, but otherwise the period of storage made little difference. The degree of lymphocytic reaction was less than with fresh homografts but there was marked infiltration with macrophages. Proliferation of Schwann cells and break-up of myelin occurred much as in fresh grafts Regenerating fibres were found to penetrate the stored grafts, sometimes reaching further than with fresh homografts of the same age, the fibres were, however, relatively few in number and abnormally thick.

Gutmann and Sanders allowed the grafts to remain in the hosts for much longer periods, and studied motor and sensory recovery in addition to making histological observations They found that the spreading reflex returned on average a little sooner than with fresh homografts, taking from 61 to 117 days, and that eventually motor recovery was distinctly more complete than with fresh homografts though not quite as good as with autografts Complete return of sensation was present in one animal after 174 days, of five others tested after 200 days two showed partial recovery and three no recovery at all. Grafts examined histologically after 200 days in the host were found to be well vascularized, and contained numerous medullated fibres varying considerably in diameter No lymphocytes were seen, and the gross macrophage infiltration characteristic of grafts removed after 25 days (*v. supra*) was no longer present, although a few macrophages and fibroblasts were seen at the junctions. Occasional patches of fibrosis were found in the grafts.

Nageotte (1917a) used homografts of nerve segments which had been fixed in 90 per cent alcohol, stored subsequently in 50 per cent alcohol, and washed thoroughly in Ringer's solution before use, to repair gaps in the sciatic nerves of dogs and rabbits. He reported recovery of normal gait and an absence of trophic ulceration in the recipients, and attributed these findings to re-innervation of the grafts. A little later Huber (1920) reported re-innervation of the grafts, and some recovery of muscle function, after using alcohol-stored homografts in rabbits. More recently, however, Sanders and his colleagues have reported unfavourably on this method of storage. Sanders and Young (1942) found that alcohol-stored grafts examined after 15 days in the host were never properly united and never vascularized, but were always enclosed in a thick vascularized capsule strongly adherent to surrounding tissue. The grafts were gradually replaced by a mass of macrophages and fibroblasts Nerve fibres sometimes reached the distal stump by traversing this mass of cells but did not penetrate the grafts themselves. Gutmann and Sanders (1942) found return of the spreading reflex in two rabbits 126 and 142 days after insertion of 2 cm. alcohol-stored homografts in the peroneal nerve, but no return in two others, and no return of sensation on the dorsum of the foot in any of these animals after 200 days. Histological examination revealed irregular bundles of nerve fibres with Schwann cells in a mass of connective tissue. Some fibres reached the distal stump but many failed to do so. Gutmann and Sanders considered that the histological findings, and such motor recovery as occurred, were just what would be expected after unaided union, and they surmised that Nageotte (1917a), who studied only transverse sections, mistook similar findings for re-innervation of the graft. Anokhin (cited by Propper-Grash-

chenkov 1912) found motor and sensory recovery in dogs after inserting homografts which had been preserved in 15 per cent formalin for 10 to 15 days but in the absence of details it is difficult to assess the validity of this claim

Weiss and Taylor (1943) studied freeze dried homografts in rats and subsequently Weiss (1943b) performed similar experiments in cats and monkeys In rats and cats the grafts became re innervated the regenerating fibres attained a satisfactory calibre and became medullated and full functional recovery was obtained as a rule about six months after inserting 1 2 cm grafts Some excellent results were also obtained in monkeys but the findings were less uniform and there were some failures which were however attributed to technical errors All the grafts were spliced in place by arterial sleeves using the technique described by Weiss (1943a) and this was thought to have assisted greatly in maintaining the fascicular topography of the nerve Sanders (1951a b) has suggested however that the shortness of these grafts may have been responsible for their success (*see* p 332) and has reported that 5 cm freeze dried homografts in rabbits behave like fresh homografts of the same size

Clinical Observations

Rehn and Hasslauer (1916) and Hohmann and Spielmeyer (1917) used nerve homografts stored at ice box temperature by Bethe s method (p 333) in a total of 18 cases but all were unsuccessful

Fisher (1919) reported excellent recovery after bridging a 1½ inch defect in the median nerve with a homograft which had been dropped into a dish of methylated spirit but it is uncertain whether the period of immersion was long enough for the spirit to affect more than the surface of the graft More recently Propper Grashchenkov (194) reported excellent results with nerve homografts stored in a 20 per cent formalin but unfortunately gave no detailed reports on his cases Klemme Woolsey and DeRezende (1943) used homografts which had been stored in formalin and subsequently soaked in water for two days and then in 75 per cent alcohol for a further two or three days in three patients The first showed good motor and sensory recovery and in the second function was beginning to return while the third case had not been under observation for sufficiently long to permit any assessment to be made In view of the observations on chemically fixed nerve homografts in animals (p 334) however Seddon (1947) and Sanders (1951b) have concluded that further trial of grafts of this type in man would be unprofitable

Schabadasch (1944) treated nerve homografts by removing the lipoid and then impregnating them with glucose and sodium glycerophosphate owing to the short period of observation however the value of this procedure cannot be assessed

Freeze dried homografts do not appear to have been used clinically as yet

Fresh Heterografts

Animal Experiments

There have been numerous histological studies of nerve heterografts in animals These have been concerned firstly with the degenerative changes occurring in and the cellular reaction evoked by heterografts and secondly with the question of whether heterografts provide a suitable *milieu* for the growth of host fibres

Huber (1895) and Marcabruni (1911) stated that a process similar to Wallerian degeneration occurred in heterografts in dog hosts Merzbacher (1905) on the other hand found that heterografts in several different species necrosed without showing the characteristic features of Wallerian degeneration Merzbacher s findings *might conceivably* have been due to the fact that his grafts were transplanted heterotopically

Further observations with orthotopic heterografts by Ingebrigsten (1915), Cajal (1928), and Sanders and Young (1942) have shown however that the histological changes which occur differ markedly from those seen in autografts and homografts; in particular, proliferation of Schwann cells does not occur in grafts of cat, dog or rat nerve in rabbits, though it may occur to a limited extent in rabbit nerve grafts in dogs.

The extent to which host fibres penetrate heterografts has been investigated by Assaky (1886), Huber (1895, 1920), Marinesco (1907), Ingebrigsten (1916), Forssman (1917), Blondin (1928), Sanders and Young (1942), and others. Huber (1895), in his earlier experiments, found host fibres in heterografts (cat to dog) after 37 days, and Blondin (1928) subsequently demonstrated host fibres in both the graft and the peripheral stump nine months after grafting segments of rabbit nerve into the sciatic nerves of dogs. with these two exceptions, however, there has been general agreement that, while fibres may reach the distal segment of the host nerve by passing outside a heterograft, they rarely penetrate the graft, and those which do penetrate extend only for very short distances.

Return of electric excitability 11 days after grafting pieces of dog nerve into the sciatic nerves of hens was reported by Gluck (1880), but Johnson (1882) was unable to confirm this finding and in the light of later knowledge it is frankly incredible. We may also feel sceptical about the claim of Huber (1895) that cutaneous sensation to pin prick returned in a dog 39 days after heterografting, and agree with Sanders (1942) that Huber's observations of motor recovery are "not sufficiently well documented to exclude trick movements." On the other hand there would seem to be no compelling reason for rejecting the report of Blondin (1928), whose animals were studied by the neurologist Alajouanine, that a return of electric excitability could be demonstrated 9 months after grafting rabbit nerve into the dog sciatic.

Clinical Observations

There have been a few reports of functional improvement after the insertion of fresh nerve heterografts in man (Robson, 1896; Sherren, 1906; Gosset, 1923; André-Thomas and Petit-Dutaillis, 1930). These cases are however not adequately documented, and it seems clear, as Sanders (1942) has concluded, that while some recovery may be possible, heterografting in man is usually followed by no recovery at all.

Stored Heterografts

Animal Experiments

Heterografts of nerve segments stored in a viable state at either 37°C. (Eden, 1919) or ice box temperatures (Duroux, 1911; Huber, 1920), have been tried, but in no instance has there been any evidence of functional recovery.

Alcohol-fixed heterografts of calf nerve in dogs, according to Nageotte and his colleagues (Nageotte, 1917c, 1919b; Guyon, Nageotte and Tournay, 1920; Guyon, 1921), become re-innervated and result in a return of electrical excitability and voluntary movement; it appears from the work of Sweet (1929), however, that any return of function which occurs is due not to re-innervation of the grafts but to fibres growing around them. Alcohol-fixed heterografts in rabbits have proved completely unsuccessful (Huber, 1920; Guyon, Nageotte and Tournay, 1920).

Heterografts of rabbit or cat spinal cord, fixed and stored in formalin, have been reported by Gosset and Bertrand (1938) to become fully re-innervated, and to lead to the recovery of normal chronaxie in the paralysed muscles, when used to bridge gaps in peripheral nerves in dogs. Sanders and Young (1942), however, found that a segment of rat spinal cord, prepared in the same way and grafted into the peroneal nerve of

a rabbit, failed to unite with either stump or to become re-innervated, and they have criticised the work of Gosset and Bertrand on the ground that recovery of sensation and muscle function were not adequately studied

Clinical Observations

Alcohol fixed nerve segments from calves and other animals, prepared according to the method of Nageotte (1917a, c, 1919b), have been used quite extensively as heterografts by French and Italian surgeons including Sencert (1918), Walther (1919, 1920) Jabier (1920b), Gosset and Charrier (1922) and Delagenière (1924) Out of a total of 112 cases collected from the literature by Sanders (1912), 26 were reported to show some improvement and 126 cases were admittedly complete failures Many of these cases are poorly documented, but it seems clear that the chances of obtaining any improvement with grafts of this type are small, and probably any improvement which does occur is due to fibres growing down around the grafts

Segments of spinal cord from rabbits and cats fixed in formalin were used clinically as heterografts by Gosset and Bertrand (1937, 1938) in 27 cases They considered that only four patients had been followed for a sufficient length of time to be assessed, and reported evidence of recovery in each of them but the validity of the criteria on which this claim rests is questionable (*see* Sanders 1912) Bjorkesten (1918) used grafts of this type from cats or sheep in 23 patients In six the graft sequestrated and in none was there any definite evidence of functional recovery In three patients the graft was removed at a subsequent operation and showed no sign of re-innervation by host fibres

FREE NERVE GRAFTING IN PRESENT DAY SURGERY

Autografts

Nerve autografting may, if certain conditions are observed, give results not inferior to those seen after well executed suture (Seddon, 1951) its application is limited however by the difficulty of obtaining autologous nerve segments in sufficient quantity without serious harm to the patient

Selection of Donor Nerve and Type of Graft

In choosing a donor nerve the following considerations should be borne in mind

Firstly the total area of cross section of the graft or grafts should be at least equal to that of the damaged nerve Single cutaneous nerve segments* are used to repair nerves of small calibre such as the digital nerves and the facial nerve, and to repair nerves of larger calibre which have been partially divided Multiple cutaneous nerve segments are used in the form of cable grafts to repair defects involving the whole thickness of large calibre nerves (Fig 105)

Secondly, as Sunderland and Ray (1947) have pointed out, the donor nerve should be one which is constant in position, readily accessible through an incision which does

* As we have seen the use of cutaneous nerve grafts to repair mixed and motor nerves has been objected to on theoretical grounds but has proved satisfactory in practice



Fig 105 A cable nerve graft composed in this instance of four strands of cutaneous nerve

not involve a pressure area, and of adequate length proximal to or between its branches. The funiculi should be large, though not so large as to interfere with vascularization, tightly packed and parallel, and the constituent fibres should be as large in diameter as possible. The resulting sensory defect should be minimal in quality and extent, and should occur in an area where cutaneous innervation is not of great importance.

Finally, a cable graft should be composed of strands of equal length, each running the full length of the graft.

Sunderland and Ray (1947) compared five cutaneous nerves—the medial cutaneous nerve of the arm, the medial cutaneous nerve of the forearm, the superficial radial nerve, the lateral cutaneous nerve of the thigh, and the sural nerve—in the light of these criteria, and concluded that the superficial radial and sural were the most suitable donor nerves. The saphenous nerve has also been used to provide material for digital nerve and cable grafts (D'Aubigné, 1946, Seddon, 1954) but leaves an undesirable area of anaesthesia, and the great auricular nerve with three of its branches has been used to reconstruct the facial nerve after resection of parotid tumours (Conley, 1955). Seddon (1954) has urged that the superficial radial nerve should not be used to repair the median unless the radial itself is irreparably damaged, because if the radial nerve on the injured side is used the patient loses such benefit as would result from sensory overlapping of the radial and median areas, while if the opposite superficial radial nerve is used the patient will have two subnormal hands instead of one.

A main nerve which has been damaged so extensively that repair by suture is impossible may be used to provide a graft to repair another nerve, and occasionally it is justifiable to sacrifice a main nerve which could be repaired by suture or even an uninjured main nerve to enable a functionally more important nerve to be repaired. Thus when the ulnar and median nerves are extensively damaged the ulnar is often sacrificed to permit repair of the median by either a free or pedicle (p. 341) graft, and when both main divisions of the sciatic nerve are severely damaged the lateral popliteal nerve may be sacrificed to provide a graft for the medial popliteal. A somewhat similar procedure which is occasionally indicated is to sacrifice some of the funiculi of a nerve to provide a graft with which to repair other funiculi of greater functional importance.

Complete division of the radial nerve, especially if suture is impossible, is best dealt with by tendon transfer (Chapter 15), and the results of this operation are so satisfactory that Seddon (1954) has gone so far as to suggest that it might even be justifiable to take a segment from an intact radial nerve for repair of a gap in the median.

There has been a good deal of discussion about the risk of avascular necrosis in large calibre grafts. There appears to be little danger with upper limb nerve segments, but it may well be, as Seddon (1954) has suggested, that the poor results obtained with grafts from the lateral popliteal nerve are due to this cause.

Predegenerated grafts as a rule appear to offer no advantages, and degeneration of long standing may actually be harmful. A possible exception may perhaps be made in the case of the lateral popliteal nerve, however, on the ground that the risk of avascular necrosis may be less in predegenerated grafts (Seddon, 1954).

Operative Procedure

Nerve grafts should lie in healthy well-vascularized tissue, and it may be necessary as a preliminary step to excise scar tissue and provide a covering of healthy skin and subcutaneous tissue by pedicle grafting (Chapter 14). If this is done the nerve should be explored at the same time so that the feasibility of repair can be determined and fu-

ture procedures planned in detail. Sometimes, when scarring is extensive, it may be preferable, however, to use a longer graft and bypass the scar rather than embark on time-consuming plastic operations (Seddon, 1954).

To allow for shrinkage the graft should be at least 15 per cent longer than the gap.

The damaged nerve is exposed and mobilized, and the stump neuromata are resected, in the same manner as for end-to-end union, except that generally speaking mobilization need not be very extensive when a graft is used unless the gap is enormous. Resection of the distal neuroma in particular should be generous, especially in digital nerves, because there may be considerable fibrosis in the distal stump which is not visible to the naked eye.

Single grafts of small calibre and cable grafts are best joined to the host nerve by the plasma clot technique (p. 324), but with single grafts of large calibre conventional sutures of very fine silk or catgut should be used (*see* Fig. 103).

Homografts and Heterografts

Homografts are much more readily available than autografts but yield such poor results that at present they should not be used. For reasons discussed earlier (p. 333), however, it seems possible that methods of obtaining satisfactory results with nerve homografts will be developed in the not very distant future.

Heterografts should never be used, and it seems unlikely that they will ever be of clinical value.

PEDICLE NERVE GRAFTING

The terminology used by different writers varies considerably. Here, however, we shall use the term *pedicle nerve grafting* to include three different procedures, distinguished by the fact that in the first the graft is obtained from the injured nerve itself, in the second some but not all of the funiculi of a donor nerve are sacrificed, and in the third a donor nerve is sacrificed completely.

Pedicle Grafting Using the Divided Nerve Only

In this type of operation, which was devised by Letievant (1873), the gap is bridged by a pedicle formed from either the proximal or distal stump of the divided nerve or by joining pedicles fashioned from both stumps (Fig. 106).

These procedures were investigated in dogs by Huber (1895), who found no histological evidence of re-innervation and no functional recovery in animals examined between 64 and 147 days after the operation. More recently Gutmann (cited by Sanders, 1942) has observed re-innervation but no evidence of functional recovery after using pedicle grafts of this type in rabbits.

In man the results have been equally discouraging. Stookey (1919) reviewed all the cases published up to 1914, numbering more than twenty, and concluded that in none was there any evidence of recovery, despite claims to the contrary by Tillmanns (1885) and MacKenzie (1909). Later writers (Frazier, 1920; Babcock, 1927) have joined with him in condemning the procedure, and it should now be regarded as obsolete.

Pedicle Grafting Sacrificing Some Funiculi of a Donor Nerve

In this procedure one or more funiculi in the donor nerve are divided in two places and the segment thus isolated is joined to the stumps of the severed nerve as shown in Figure 107. Alternatively, the stumps of the divided nerve may be inserted into lon

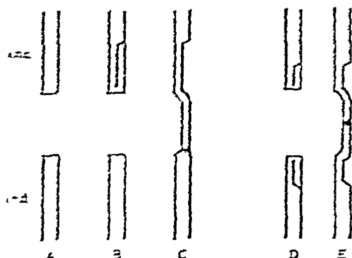


Fig. [illegible] Pedicle nerve grafting using the injured nerve. A single pedicled graft may be obtained from the central stump (i.n.c.) alone as shown in B and C, or a short graft may be obtained from both central (i.n.c.) and distal (i.n.d.) stumps as shown in D and E. (After Sanders.)

gradual [illegible] made between the funiculi of the donor nerve. This modification, termed by Sanders (1942) *double lateral implantation*,* was designed to avoid damaging axons in the donor nerve. In practice however, as Sanders (1942) has pointed out, division of some host axons is unavoidable and is probably responsible for any functional recovery which occurs.

Kilvington (1908) used part of the medial popliteal nerve as a pedicle graft in the lateral popliteal nerve in two dogs and obtained some functional recovery. He found however that electrical stimulation of either nerve proximal to the graft produced contraction of both flexor and extensor muscles, indicating either that fibres from

* [illegible] who introduced the term double lateral [illegible] to include both forms of [illegible] this paragraph.

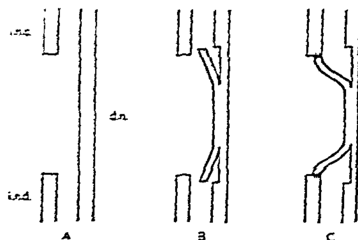


Fig. 107. Pedicle nerve grafting sacrificing some fasciculi of a donor nerve (d.n.). The central and distal stumps of the injured nerve are denoted by i.n.c. and i.n.d. respectively. (After Sanders.)

the central part of each nerve now extended into the peripheral parts of both, or else that fibres growing out of both central stumps had branched and that branches of the same fibre innervated antagonistic muscle fibres. Sachs and Malone (1922) also working with dogs, found histological evidence of axon branching and reported full functional recovery after double lateral implantation of the lateral popliteal nerve into the medial popliteal in dogs, and Ballance and his colleagues (Ballance, 1923, 1924; Ballance, Colledge and Bailey, 1926) obtained functional recovery in monkeys and cats after both double lateral implantation (in Sanders' sense) and pedicle grafting involving deliberate sacrifice of donor nerve funiculi.

Varying degrees of recovery after these operations has also been reported in clinical cases (Dziewonski, 1906; Erlacher, 1914a; Wexburg, 1917; Cahen, 1917; Souttar and Twining, 1918; Joyce, 1919, 1920). On the other hand no recovery, but instead loss of function in consequence of damage to the donor nerve, has also been observed (Stopford, 1920) and the results on the whole have not been good enough to compensate for this risk. Today, therefore, these operations are rightly regarded as obsolete.

Pedicle Grafting Sacrificing a Donor Nerve Completely

In 1947 Strange reported a case in which the ulnar and median nerves were both injured in the forearm, and in which the defect in the median was repaired by a pedicle graft from the ulnar by the two stage procedure illustrated in Figure 108. Excellent sensory recovery was obtained, but there was no return of power in the thenar muscles (Strange, 1950); tendon transfer (p. 303) was therefore performed later to restore opposition of the thumb.

Seddon (1954) has subsequently reported

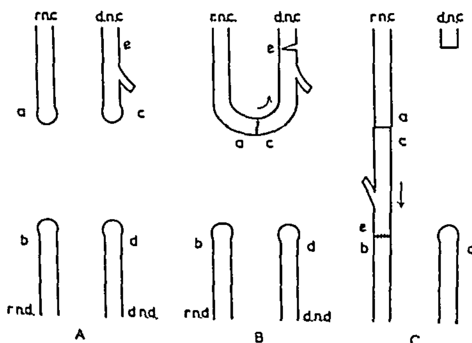


Fig. 108. Strange's operation. A, Defects in two adjacent nerves. B, First stage—blood supply to the graft at e. C, Second stage. Blood supply to the graft at c. r.n.c., central end of recipient nerve; d.n.c., central end of donor nerve; r.n.d., distal end of recipient nerve; d.n.d., distal end of donor nerve. The letters a, b, c, d, e are for identification. (Redrawn from the *British Journal of Surgery* by courtesy of the publishers and Mr. F. G. St. Clair Strange.)

four cases of simultaneous injury of the median and ulnar nerves in which this operation was used; three were described as successful and one as showing partial recovery.

This procedure should be considered only when two adjacent nerves have been damaged so extensively that neither can be repaired by suture, or when two adjacent nerves have been divided and successful repair of one is so predominantly desirable that it is justifiable to sacrifice the other nerve even though it could be sutured.* Even under these circumstances Strange's operation is not often needed because comparable results can usually be produced by using a free graft from the nerve available to be sacrificed, but occasionally, when a free graft would be liable to avascular necrosis on account of its large calibre or because it would have to lie in a bed of scar tissue, it is the procedure of choice. The graft may, of course, be obtained from either the central or peripheral stump of the nerve sacrificed.

According to Seddon (1954) this is the ideal operation when ischaemia has damaged the median and ulnar nerves in the forearm beyond hope of recovery;* it may also be indicated in the lower extremity, for example when the medial and lateral popliteal nerves are divided,† but so far does not appear to have been used in this situation.

*In comparing different nerves from this point of view one must consider not only the functions normally subserved by the nerves but the extent to which loss of these functions can be compensated for by procedures such as tendon transfer.

*The ulnar is, of course, sacrificed to permit repair of the median

†In this event the lateral popliteal nerve would be sacrificed to repair the medial.

NERVE CROSSING

There are two forms of nerve crossing: total nerve crossing (Fig. 109), in which a nerve adjacent to the injured nerve is completely divided and its central stump is sutured to the peripheral stump of the injured nerve; and partial nerve crossing (Fig. 110), in which a central stump formed by partly dividing an adjacent nerve is sutured to the distal stump of the injured nerve The procedure known as peripheral

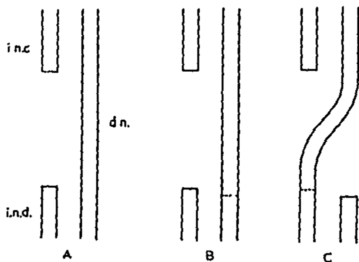


Fig 109 Total nerve crossing i n c., central stump of injured nerve. i n d., distal stump of injured nerve. d.n., donor nerve. (After Sanders)

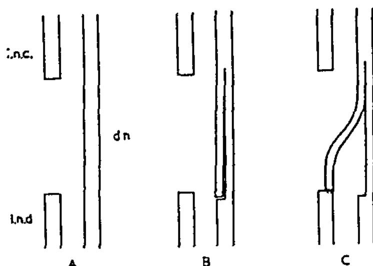


Fig 110 Partial nerve crossing i n c, central stump of injured nerve. i n d, distal stump of injured nerve d n, donor nerve (After Sanders)

stump implantation, in which the distal stump of the injured nerve is inserted into a slit made between the funiculi of an adjacent nerve, in so far as it is followed by re-innervation, is a form of partial nerve crossing because such re-innervation can occur only in consequence of accidental division of axons in the sound nerve.

History*

It has been definitely established in animals, and also in man, that after nerve crossing re-innervation can occur and paralysed muscles can regain the power of contraction.

The first attempt to study the results of nerve crossing was made by Flourens (1828), who crossed completely the two main nerves leading from the brachial plexus to the ventral and dorsal aspects of the wing of a cock, and reported that after a few months the bird could fly as well as before the operation.

Though Flourens' experiment is often quoted its significance is doubtful because the main muscles of flight acting on the humerus of the wing receive their nerve supply from branches which arise above the level at which crossing was performed (*see* Stefani, 1886; Cunningham, 1898, Sperry, 1945). Subsequently, however, return of voluntary movement or response to electrical stimulation after nerve crossing has been demonstrated in various species including dogs, cats, rabbits, pigs, rats and monkeys, by many observers including Rawa (1885), Stefani (1886), Howell and Huber (1892), Cunningham (1898), Forssman (1900), Kennedy (1901, 1914), Bethe (1905), Spitzy (1905), Kilvington (1905, 1912), Osborne and Kilvington (1910a, b), Ballance, Colledge and Bailey (1926), Ballance (1931), and Sperry (1941). Successful nerve crosses have included hypoglossal to vagus, ulnar to median and *vice versa*, radial to ulnar-median and *vice versa*, and medial to lateral popliteal and *vice versa*

Langley and Anderson (1904) tried to determine the extent to which re-innervation is contingent on similarity in fibre spectrum of the nerves crossed They found that nerves containing a preponderance of efferent fibres proceeding from the spinal cord,* when crossed to a peripheral stump supplying skeletal muscle, resulted in a return of

*In preparing this section much help has been obtained from valuable reviews by Sanders (1942) and Sperry (1945)

*i.e. motor fibres innervating skeletal muscle, and preganglionic sympathetic fibres.

electrical excitability in the paralysed muscles on stimulation via the nerve, whereas nerves containing a preponderance of postganglionic sympathetic fibres or afferent fibres did not.

Various investigators, including Kilvington (1905), Kennedy (1914), Dogliotti (1935) and Aird and Naffziger (1939), have joined the proximal stump of one nerve to its own and one or more additional distal stumps, or alternatively part of the proximal stump to the whole distal stump of the same nerve, and have found a marked increase in the number of regenerating fibres below the suture line, and a return of function in excess of that originally and normally mediated by the proximal nerve fibres *

In man, nerve crossing has been used mainly in the treatment of facial paralysis. The first successful case appears to have been that of Faure and Furet (1898), and thereafter many others have been reported (Manasse, 1900, Barrago-Ciarella, 1901, Frazier and Spiller, 1903, Cushing, 1903; Gluck, 1903, Korte, 1903, Ballance, Ballance and Stewart, 1903, Taylor and Clark, 1904, Sherren, 1906, Stookey, 1922; Ballance 1923, 1924 Adson, 1925; Ballance and Duel, 1932, Hanrahan and Dandy, 1944). The nerves used to provide the proximal stump include the spinal accessory and its motor branch to the trapezius and sternomastoid muscles, the hypoglossal, and the descendens hypoglossi. Both total and partial crossing has been tried, the former giving the better results. In an attempt to overcome the deformity resulting from paralysis of the sternomastoid and trapezius muscles after total crossing of the spinal accessory to the facial, the descendens hypoglossi has been joined to the distal stump of the spinal accessory as an additional procedure in some cases. When nerve crossing has been used clinically to treat lesions of nerves other than the facial the functional results have usually not been very good, but there has been definite evidence of re-innervation in some cases (*see* Sherren, 1906; Spitzy, 1907; Maragliano, 1911; von Hacker, 1914a; von Hofmeister, 1915; Kölliker, 1917, Sperry, 1945).

The extent to which normal co-ordinated voluntary movement of muscle groups is regained after nerve crossing has been much debated.

Inevitably, after this operation, there is an extreme amount of "hetero-innervation" (Sanders, 1942), every re-innervated motor unit becoming connected with a different fibre from the one which supplied it originally In consequence, unless some process of re-adjustment occurs, movement is likely to be unco-ordinated. Thus, in the first place, the re-innervated muscle may contract when relaxation would be more in accord with the general pattern of movement, or *vice versa*. Secondly, *associated movements* tend to occur, contraction of the muscles newly innervated by fibres from the donor nerve being accompanied by contraction of the synergists of the muscles supplied by these fibres before the operation. Thirdly, owing to axon branching in the re-innervated nerve, muscle fibres in different muscles may be innervated by branches of the same axon, and in consequence contraction of one set of fibres cannot occur without contraction of the other set, a phenomenon which is termed *mass movement*.

Much work has been done with the object of finding out whether associated movements occurring after nerve crossing can ever become dissociated, and if so how this dissociation is brought about.

Rawa (1885), and Howel and Huber (1892), claimed that complete dissociation of the associated movements took place. Cunningham (1898) challenged the validity of this conclusion on the ground that it was

*It was therefore suggested by Dogliotti (1935) and by Aird and Naffziger (1939) that function might be improved in selected patients with paralysis of some but not all of the muscles supplied by a particular nerve as the result of poliomyelitis, by dividing and immediately suturing the nerve in question

based on experiments in which the nerves crossed (e g the median and ulnar) were ones which supplied synergic groups of muscles, and that in consequence associated movements would be difficult to recognize He suggested that a more crucial experiment would be to cross nerves such as the radial and median, or radial and ulnar, which supply antagonists, and showed experimentally that when this was done associated movements could be clearly recognized and persisted apparently indefinitely

In spite of Cunningham's work, however, it was widely accepted that inco ordination due to association of movement after nerve crossing in animals was only temporary, and that eventually normal co ordinated movements were re established by a process of readjustment or "re education" Attempts were therefore made to elucidate the nature of the re education process, and to determine the level at which it occurred, by either cortical stimulation or lesion methods (Kennedy, 1901, 1912, 1914, Osborne and Kilvington, 1910a, b, Ballance, 1931, Barron, 1934, Anochin and Iwanow, 1936, and others), but the more recent investigations of Weirous and Olmsted (1941) and Sperry (1941, 1945) have cast doubt on the validity of the conclusions which were drawn, and would seem to indicate "that re educative neural adjustments, like those of learning under normal conditions, are confined to higher brain relations, and never effect any switching of basic neuron associations of the spinal cord or of the primary sensory and motor nuclei of the brain" (Sperry, 1945)

In man, as in lower animals, mass and associated movements occur after nerve crossing By developing trick movements some of the disability can be overcome, but again it appears from critical examination of the reported cases that there is no clear evidence that dissociation ever occurs

On the sensory side, as Sperry (1945) has expressed it, "afferent excitations are in part projected to the cerebral cortex, but are also short circuited through spinal, cerebellar, thalamic, and other lower levels," and "the possibilities of re education vary greatly, according to the nature of the central and motor effects which are produced by the afferent stimuli concerned" Adaptive adjustment of overt voluntary reactions to compensate for certain simple derangements of sensation can occur not only in man but also in the rat (Sperry, 1943), on the other hand the possibility of adaptive adjustment at lower levels, or, at the other extreme, of *recovery of refined discriminatory sensory* functions, seems very remote

Nerve Crossing in Present Day Surgery

Nerve crossing, despite its disadvantages, is still useful for the treatment of lesions of the facial nerve proximal to the geniculate ganglion, such as occur in association with acoustic nerve tumours and operations for their removal For division of the trunk of the facial at a lower level, such as occurs sometimes during mastoid operations, end-to end anastomosis or autografting gives better results if skilfully performed, but is technically more difficult *

Total crossing of the hypoglossal to the facial, commonly known as hypoglossal-facial anastomosis, is the best of the nerve crossing operations for facial paralysis. McKenzie and Alexander (1950) found that in three quarters of their cases the face appeared symmetrical at rest and also on quiet smiling, though not when the patient laughed heartily There was usually good movement during eating and talking but sometimes it was excessive In spite of paralysis of half of the tongue speech was normal The operation in most of these cases had been performed two to six weeks after division of the nerve, but an excellent result

* An alternative procedure is to correct the deformity as far as possible by means of fascial slings (Chapter 15) or pedicled muscle transplants (Chapter 16), and make no attempt to restore continuity of the nerve.

was obtained in one patient operated on after a lapse of two and a half years

Crossing of the spinal accessory nerve to the facial is much less satisfactory and should be abandoned. Mass and associated movements are pronounced, and in addition there is usually a considerable degree of drooping of the shoulder and loss of function due to paralysis of the trapezius muscle.*

Anastomosis of the descendens hypoglossi either to the facial or to the distal stump of the hypoglossal after hypoglossal-facial anastomosis appears to be of little or no value.

Nerve crossing for lesions of nerves other than the facial is now obsolete.

*The trapezius is innervated partly by the spinal accessory nerve and partly by the third and fourth cervical nerves. The consequences of sacrificing the spinal accessory depend on the extent to which the muscle is supplied by this nerve.

CHAPTER 18

Transplantation of Bone

Written in collaboration with Norman W. Nisbet

STRUCTURE AND FUNCTION OF BONE

Macroscopic Structure

Bone is a specialized connective tissue whose characteristic hardness is due to a dense matrix consisting of collagenous fibres embedded in a *mucopolysaccharide ground* substance impregnated with lime salts Its hardness belies its true nature, for in reality bone is a highly cellular and vascular tissue, whose components are constantly changing

Macroscopically two types of bone may be distinguished in the normal adult skeleton *compact (or cortical) bone* and *cancellous (or spongy) bone* In general the outer part of a bone is formed of compact bone, and the inner part of cancellous bone, but the relative amounts of each type differ in different bones and in different parts of the same bone The difference in texture is due to a difference in porosity, which in turn depends on a difference in the vascular pattern Even cortical bone is porous, but the pores are invisible to the naked eye, whereas cancellous bone is composed of interlacing trabeculae with large vascular cavities in between.

During life bone is permeated by blood vessels and, except where it is covered by articular cartilage, is surrounded by a fibrous membrane called the *periosteum* In the interior of a long bone there is a cavity, termed the *medullary cavity*, containing bone marrow and lined by a vascular membrane, the *endosteum*, which has a single layer of osteogenic cells

Microscopic Structure

In ordinary decalcified histological preparations viewed at moderate magnifications mature compact bone appears to be composed of a series of elements known as *Haversian systems*, each consisting of concentric lamellae surrounded by a central canal, the *Haversian canal* The number of lamellae varies from four to twenty, but is usually less than six (Ham, 1953) In a long bone the Haversian systems are directed mainly in the longitudinal axis, and so are best seen in transverse sections (Fig 111)

The intervening areas between the Haversian systems are filled with *interstitial lamellae* These are the remains of older Haversian systems which have been eroded and destroyed in the remodelling process to which bone is constantly subjected John Hunter (1772) discovered this process by observing the pink staining of the growing bones in pigs and other animals fed on madder *Concentric lamellae* are found on the superficial and medullary surfaces, and encircle the entire shaft, thus binding the bone together

It has been shown by silver impregnation techniques that the collagenous fibres of the Haversian systems form a complicated woven pattern which gives to bone a com-



Fig 111 Compact bone Transverse section showing Haversian systems. Decalcified. Haematoxylin and eosin. ×120.

bination of great resilience and strength (Gebhardt 1905, Pritchard, 1956a). Fibres pass between adjacent lamellae so that, as Pritchard (1956a) has pointed out, it is erroneous to consider the Haversian systems as built up of discrete cylinders.

According to Rouiller (1956) there are two types of lamellae, one rich in collagen and the other rich in inorganic salts and mucopolysaccharide

It must be emphasized that bone is in fact more homogeneous than is commonly thought. Indeed much compact bone has no lamellar structure, and according to Pritchard (1956a) many of the appearances seen in ordinary histological preparations are artefacts.

The Haversian or central canals are nutrient vascular channels and contain one or two capillaries. The canals vary in size, and the larger ones may contain an arteriole and a venule as well as capillaries and some connective tissue, whereas the smaller ones contain only capillaries. Their general direction is parallel to the long axis of the bone, but their course is very irregular and they branch and anastomose freely with their neighbours. Another system of canals, the *canals of Volkmann*, run at right angles to the Haversian canals; they open on the periosteal and medullary surfaces, and convey blood vessels to and from the Haversian capillary systems in the interior of compact bone (*see* Brooks and Harrison, 1957).

Cancellous bone is generally similar in structure to cortical bone, but the Haversian canals are fewer in number and occur mainly in the thicker trabeculae.

There has been much speculation about the factors which determine the architec-

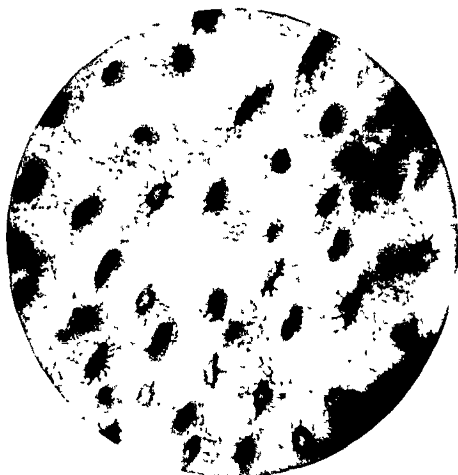


Fig. 112 Mature lamellar bone. Ground, undecalcified and unstained section showing lacunae and branching canaliculi. ×250

tural arrangement of a bone (*see* Koch, 1917, Dawson and Spark, 1928; Murray, 1936, Cowdry, 1950, Clark, 1952, Maximow and Bloom, 1957). Wolff (1899) believed that the internal structure of a bone was determined by external stresses which acted upon it, but this view has not passed unchallenged (Jansen, 1920, Pritchard, 1956a).

Bone contains a variety of specialized cells. The characteristic cell of mature lamellar bone is the *osteocyte*, a flattened cell having numerous branching processes. The osteocytes occupy spaces in the bone called *lacunae*, from which fine branching channels called *canaliculi* radiate in all directions and communicate freely with neighbouring lacunae (Fig. 112), with Haversian and Volkmann canals, and with the surface of the bone. In living bone the cells completely fill the lacunae, but in ordinary histological preparations they do not appear to do so. The fine processes of the osteocytes *occupy the canaliculi, and there appears to* be continuity between the processes of adjacent cells of the same Haversian system; it seems therefore that these cells form a true syncytium (Clark, 1952; Howard, 1956). The canaliculi allow the transfer of nutrient and waste materials to and from the osteocytes.

Much remains to be learned about the function of osteocytes, but they are unquestionably living cells (Cowdry, 1950), and are an essential constituent of bone (Pritchard, 1956b). They play a vital part in the metabolism of bone, and when the osteocytes die the bone is removed and replaced. They are moreover undoubtedly concerned in the



Fig 113 Young lamellar bone and periosteum The cells incorporated in the lamellae are osteocytes, those on the surface of the lamellae and lining the inner osteogenic layer of the periosteum are osteoblasts. Van Gieson. ×150.

constant remodelling which takes place in bones during life

The precursor of the osteocyte is the *osteoblast* Osteoblasts cover all bony surfaces and line the inner aspect of the periosteum (Fig 113) In ordinary histological preparations they appear as inert flat cells (Goodsir and Goodsir, 1845, Collins, 1949; Clark, 1952) They leap into life when bone is fractured or inflamed, and immediately start the healing process. Very little is known about the physiological factors which initiate their proliferation (Harris, 1954; *see also* Pritchard, 1956b) When osteoblasts become imprisoned by the formation of bone they become osteocytes. The preservation of the vitality of the cells in an enclosed rigid tissue is one of the remarkable properties of bone and reflects its plasticity (Ham and Harris, 1956).

There is a difference of opinion as to whether osteoblasts develop from a special race of cells resident in the skeleton or, alternatively, whether cells capable of differentiating into osteoblasts are widely distributed throughout the connective tissues. Observations on the behaviour of explants of bone in tissue culture (Strangeways and Fell, 1926, Fell, 1932, 1935, 1939, 1951, 1956; Gaillard, 1942, 1953) and bone transplants have so far not succeeded in resolving this matter.

Osteoblasts lay down bone matrix (Weinmann and Sicher, 1955; Amprino, 1956; Belanger, 1956; Jackson and Randall, 1956), and probably also secrete alkaline phos-

phatase and the hexose phosphate and mucopolysaccharide substrates required for calcification of the matrix. They thus play an essential part in the formation of bone, and without them this process cannot take place.

Wherever bone is being absorbed large multinucleated cells called *osteoclasts* are often found. These were discovered by Koelliker (1873) and have subsequently been studied by many investigators including Arey (1920), Jordan (1925), Kirby Smith (1933), Heller, McLean and Bloom (1950) and Hancox (1956). It is not yet settled whether they play a vital role in bone resorption.

The periosteum is composed of an outer layer of white fibrous connective tissue with a few elastic fibres, and an inner or cambium layer containing cells capable of differentiating into osteoblasts (Clark, 1952; Weinmann and Sicher, 1955). From the deep aspect of the periosteum fibres known as *Sharpey's fibres* run at right angles into the bone at regular intervals, especially near the epiphyseal ends of long bones, and seem to have the function of nailing together the superficial lamellae. Tendons gain attachment to bone by perforating it in much the same way. The thickness of the periosteum differs in different parts of the skeleton and also with age. In children the periosteum is normally thick, whereas in the elderly it is thin, friable and less cellular, and its layers are not sharply defined. The periosteum is highly vascular and is supplied with somatic nerves so that it is sensitive to pain.

The endosteum, which lines the marrow cavity and covers the bony trabeculae, consists of a single layer of cells which may be osteogenic or osteolytic or a mixture of both, with very little intercellular substance.

Blood Supply of Bone

Bone derives its arterial supply from several sources, including the vessels of the periosteum, the periarticular plexus and the nutrient artery. Vessels from the periosteum penetrate the cortex by the canals of Volkmann and anastomose with the vessels of the Haversian systems. Vessels from the periarticular plexus supply both the epiphysis of a long bone and the metaphysis, and in most animals and man there is little or no communication between these two vascular territories (Harris, 1929; Clark, 1952; Trueta and Harrison, 1953), though according to Brooks and Harrison (1957) the upper end of the femur in the rabbit is exceptional in this respect. The nutrient artery, which is usually single, penetrates the cortex obliquely and ramifies in the marrow, where it divides into ascending and descending branches. After supplying the marrow and endosteal plexus these vessels end in the metaphyseal anastomosis.

Bones possess a very free venous drainage (Begg, 1951; Rutishauser, 1956). Many veins pass directly through the shaft of a bone from the medullary cavity to the periosteum.

If the blood supply to compact bone is examined in detail some curious anomalies will be found. A Haversian canal for example may contain only a single vessel, which may be either an artery or a vein (Harris and Ham, 1956), or it may contain both an arterial and a venous channel. The varied vascular pattern is probably associated with the remodelling of bone.

There has been much discussion about the lymphatic drainage from bone. It is now generally believed that lymphatic vessels run in the Haversian canals and also in the bone marrow, though Pritchard (1956a) says that the presence of lymphatics in bones is doubtful.

Chemical Composition and Crystalline Structure

The organic matrix of bone, consisting of collagen together with a small amount of mucopolysaccharide cement substance, com

prises about 35 per cent of bone. Electron microscopy suggests that the collagen of bone is very similar to, if not identical with, the collagen of ordinary connective tissue, although as Neuman and Neuman (1958) have suggested, fundamental chemical differences probably exist between collagens from different sources. The inorganic material, which accounts for approximately 65 per cent of the dry weight of bone, consists mainly of calcium phosphate. It is widely accepted that this substance occurs in the form of a hydroxyapatite (Carlström, 1955, Trautz, 1955, Carlstrom and Engstrom, 1956; Neuman and Neuman, 1958), though this has been denied by Posner and his colleagues (Dallemagne, Fabry and Posner, 1954, Posner, Fabry and Dallemagne, 1954, Dallemagne and Fabry, 1956), who refer to the bone salt as a "pseudo apatite."

A great deal of work has been done in recent years on the ultra-structure of bone by means of polarization microscopy, X-ray diffraction, electron microscopy, and studies using radioactive isotopes Schmidt (1947), for example, by examining thin sections of bone with the polarizing microscope concluded that the collagenous fibres of bone were formed first and had a directing influence on the deposition of mineral salts, which were laid down with the long axes of the crystals parallel to those of the collagen, and Robinson and others (Robinson, 1952 Robinson and Watson, 1952, Engstrom, 1953, 1956, Ruth, 1953; Watson and Robinson, 1953, Robinson and Watson, 1955, Carlstrom and Engström, 1956, Jackson and Randall, 1956, Robinson and Cameron, 1956), using shadow casting in conjunction with electron microscopy, observed minute tabular bone crystals of regular size and shape arranged on or in the collagen fibres. The crystalline structure of bone furnishes enormous surfaces for ionic exchanges (Hendricks and Hill, 1950).

The equilibrium between the bone crystals and the extracellular fluids is believed to be controlled by parathormone (Howard, 1956). It has been suggested that the crystals may be sensitive to mechanical forces, and this would help to explain the physiological response of bone to mechanical influences (Robinson, 1952).

Although the microcrystalline structure of bone is remarkably constant it seems likely that the crystals are continuously replaced; in the rat, for example, Hevesy, Levi and Rebbe (1940) found that radioactive calcium and phosphorus were removed and replaced in three weeks In consequence adventitious ions, including harmful radioactive isotopes, may become incorporated, and in some instances retained for long periods (Dallemagne and Fabry, 1956; Engström, 1956. *See* also Neuman, 1956).

Metabolism of Bone

Bone cells have been described by Cameron (1954) as "highly strung" mechanisms requiring a generous supply of oxygen, salts, amino-acids and other substances. Osteocytes are influenced by parathyroid, thyroid, and pituitary hormones; osteoblasts by oestrogens and androgens; and osteoclasts by parathyroid hormone. Vitamin C is required for the formation of collagen and thus has an important influence on the formation of bone matrix. Vitamin D is required to complete the process of calcification, and Vitamin A also has an important influence on the nutrition of bone, lack of this substance causing a proliferation of osteoblasts and excess causing osteoclastic activity. Osteoblasts are peculiarly sensitive to deficiency of plasma protein. Full accounts of the many controlling influences on bone have been published in a variety of symposia and monographs (Miner, 1955; Bourne, 1956; Wolstenholme and O'Connor, 1956).

Although bone is primarly a supporting tissue it performs other important functions

associated with the metabolic processes of the body The skeleton contains approximately 97 per cent of the total body calcium and this supply can be rapidly mobilized to maintain a constant level of calcium in the blood wide variations of which are incompatible with health (Bauer Aub and Albright 1929) Bone thus acts as a buffer system in the stabilization of the blood calcium (Howard 1956 Neuman and Neuman 1958)

FORMATION AND GROWTH OF BONE

Early Experiments

The growth of bones was studied in animals and birds by Hales (1727) Duhamel (1743) and John Hunter (1772) who used markers consisting of either drill holes or pieces of lead shot embedded in the bone They found that the markers did not separate as the bone grew in length and were thus led to the conclusion that longitudinal bone growth was not due to interstitial enlargement but occurred at the epiphyses

Belchier (1736a b) drew attention to the pink staining of bone which occurs in animals fed with madder and this phenomenon which is an example of vital staining was utilized by Duhamel (1743) to study the mechanism by which bones increase in girth (*see* Keith 1919 Sissons 1956) Duhamel concluded that the increase in girth was due solely to production of bone by the periosteum but Haller (1767) who repeated Duhamel s experiments concluded that the periosteum was non osteogenic and that bone formation was a function of the arteries

Many other experiments were performed with madder feeding in a variety of animals and birds (Cameron 1930) but little new knowledge was added until John Hunter made the fundamental discovery of the remodelling processes of accretion and absorption and was thus able to explain how bones could increase in size while retaining their shape

It may be remarked that madder is still used in the study of osteogenesis though as a rule the active principle of the dye in the form of alizarin red S is employed and is injected subcutaneously or intraperitoneally (Payton 1932 1933 Brash 1934 Hoffman and Schour 1940 Bourne 1956) Brooks (1917) and Thurlow and Macklin (1918) studied the healing of bone transplants by this method Jarabak (1951) Myers (1955) and Leblond and Greulich (1956) used alizarin in combination with radioactive isotopes in the study of the mineralization of bone and found that the uptake of radiocalcium was very similar to that of alizarin Hoecker (1956) found that radium and alizarin were deposited immediately in growing bone

There has been much disagreement concerning the osteogenic capacity of periosteum This question began to be of surgical importance towards the end of the 18th century with the development of the operation of excision of diseased joints (White 1769 Park 1783 Moreau 1816 Syme 1831) because surgeons wanted to know whether preservation of the periosteum was necessary to obtain sound bony union

Duhamel (1743) held that periosteum was osteogenic and Syme (1831) and Ollier (1867) following experiments in dogs rabbits and other species concluded that the periosteum was not only osteogenic but was essential for osteogenesis Ollier reported further that the osteogenic power of the periosteum varied between different species and between young and adult animals he also put the matter to the test in surgical practice and found that after performing diaphysectomy for osteomyelitis a new shaft

regenerated from the periosteum. On the other hand Macewen (1912), who repeated Syme's experiments on dogs and other animals, could assign no osteogenic function to the periosteum. He held that osteoblasts escaped into the tissues and the blood stream on laceration of the periosteum, and believed that these cells came from inside the bone.

Keith (1919) has attributed the discrepancy to the fact that Syme and Ollier used sharp dissection, and thus stripped the whole periosteum including the osteogenic cells in the deeper layer, whereas Macewen used blunt dissection and stripped only the fibrous part of the periosteum. It seems doubtful whether this can be the whole explanation, but whether it is or not there is now little doubt that the periosteum is osteogenic in its proper sphere, which is in the environment of the skeletal system (Clark, 1952; Pritchard, 1956b). Its power of osteogenesis diminishes with increasing age, however, and normally the periosteum forms bone only in children, or when bone has been injured or is diseased.

Current Views on Osteogenesis

It is useful to distinguish between *orthotopic osteogenesis*, i.e. osteogenesis occurring in the skeleton or in the tissue from which the skeleton is formed during normal development, and *heterotopic osteogenesis*, i.e. osteogenesis in other sites.

Orthotopic Osteogenesis

During normal development of the skeleton two forms of ossification may be distinguished: ossification in membrane and ossification in cartilage. Orthotopic ossification also occurs in post natal life during the repair of fractures and in various diseases of bone. The histological changes which occur have been studied intensively, and have been described in detail by Ham (1953) and others.

Although theories of ossification have been suggested in which the cells play little or no part (*see* e.g. Leriche and Policard, 1928), it is now, as we have seen, generally agreed that the process is dependent on the activity of osteoblasts. These cells form bone matrix, consisting of collagen fibres and amorphous ground substance, and calcium salts are deposited in the ground substance. It is generally believed that, while it may take some time for completion of mineralization to occur (Logan, 1940), the process begins as soon as ground substance is formed (Bloom and Bloom, 1940; McLean and Bloom, 1940; Urist and McLean, 1941a, b; McLean and Urist, 1955), though recently Lisco (1956) has challenged this view.

The deposition of bone salt is a complex process, and the chemical mechanisms involved have not been fully elucidated. It seemed until recently that the key to an understanding of the process had been found when Robison (1932) put forward his phosphatase theory, according to which some unidentified phosphate ester is hydrolized by one of the phosphatase enzymes liberated by osteoblasts (and possibly other cells) to produce a sufficiently high concentration of phosphate ion to cause precipitation of bone salt.

Robison's theory assumes that the extracellular fluids are nearly saturated with respect to bone mineral, and that a slight increase in phosphate concentration would cause precipitation. Originally the occurrence of adequate amounts of phosphate ester was simply taken for granted, but subsequently cellular glycolysis was invoked to explain this (Harris, 1932). An alternative theory, which in recent years has tended to supplant Robison's theory, is that the collagen fibres in the bone matrix induce the formation of hydroxyapatite crystals, and determine their orientation, by a process known as *epitaxy* or crystal-seeding, and that phosphatase is concerned primarily in the formation of the matrix. According to Sognnaes (1955), however, the presence of alka-

line phosphatase in the osteogenic cells at all stages of bone formation, calcification and absorption suggests that the enzyme is concerned in the whole life cycle of the mineralized tissues. Bourne (1956) summarized the situation by stating that phosphatase is associated with the production of phosphate ions which secures the precipitation of calcium as bone salt, that it is associated with the formation of organic bone matrix and the formation of a phosphate ester for the catalytic crystallization of bone salt, and by keeping the surface of bone crystals free from ester phosphate, permits the growth of the bone crystal. The whole question is still *sub judice*, and we need not pursue it further here. For a full discussion the reader is referred to the monograph by Neuman and Neuman (1958).

Heterotopic Osteogenesis

Heterotopic osteogenesis occurs in a variety of pathological conditions, and may also be produced experimentally. When it is thought that the stimulus by which it is brought about originates in neighbouring tissue the process is sometimes referred to as *osteogenesis by induction* (p. 35).

Nageotte (1918) and Polettini (1922) observed ossification in the vicinity of alcohol-fixed bone grafts, and considered this to be due to chemical stimulation of host tissue by alcohol soluble substances liberated by the grafts, analogous to the embryonic organizers discussed in Chapter 10.

Levander (1938) produced bone in the rectus abdominis muscle of rabbits by injecting alcoholic extracts of autologous bone and callus, and his results were confirmed by Lacroix (1951), and Cohen and Lacroix (1955), who called the inducer *osteogenin*. On the other hand negative or equivocal results were obtained by Annersten (1940), Hancox (1947), and Lindahl and Orell (1951); and Heinen, Dabbs and Mason (1949) found that bone often appeared in control rabbits injected with alcohol alone.

Huggins (1931), as we have seen (Chapter 2), showed that transplants of epithelium from the urinary tract induced bone formation in dogs and other animals, and Lacroix (1917) observed bone in the vicinity of transplants of cartilage placed beneath the capsule of the kidney.

It seems almost certain that these are examples of osteogenesis by induction in the sense in which we have defined this term (*v. supra*), but the mechanism has not been elucidated.

BONE GRAFTING

HISTORY*

Animal Experiments

From 1880 to 1918

During the early part of the nineteenth century many observations were made on the fate of portions of skull removed with a trephine and *immediately replaced* as autotransplants. Wagner (cited Ollier, 1867), reviewing this work, thought that the transplants were probably absorbed and replaced by new bone, but was unable to draw any firm conclusion because he doubted the reliability of the observations. Ollier (1867), of Lyons, set out to re-investigate the matter and performed many experiments in rabbits, cats, dogs, birds and other species, on the fate of bone transplants, and on the rather more general problem of bone regeneration.

Ollier found that subcutaneous autotransplants and homotransplants of periosteum formed hard nodules, suggesting that they

*In preparing this section much help has been obtained from the review by Chase and Herndon (1955), and the bibliographies published by Bassett (1954, 1955, 1956, 1957).

had undergone ossification, but were as a rule later absorbed, though in one rabbit bone was demonstrated at the site of transplantation three years later. Heterotransplants were rapidly absorbed, and often their disappearance was accompanied by suppuration. Heterotopic autotransplants or homotransplants of whole bones, or of macroscopic pieces of bone with the periosteum attached, in rabbits—though not in dogs—often survived, became vascularized, and increased in thickness, sometimes, on the other hand, the bone proper necrosed and was eliminated, but in this event new bone was formed from the periosteum, which survived. Transplants of bone devoid of periosteum and endosteum failed to take in older animals, and even in young animals were absorbed after a time.*

Ollier also performed a few experiments with orthotopic† bone transplants and observed that bony union occurred between the transplant and the host bone. He concluded from all this work that the periosteum is essential to the success of a bone transplant, and that its function is to provide a bed (*couche*) of osteogenic tissue which plays an important part in regeneration of the transplant and, in the special case of orthotopic transplants, in establishing continuity with host bone.

Barth (1893, 1894) studied the behaviour of grafts of both living and dead bone placed in trephine openings in dogs' skulls. To the naked eye there was no difference between the behaviour of dead and living grafts when observed after a comparatively short interval (13-61 days), and Barth concluded that the whole of the substance of a compact bone graft dies, but may subsequently be replaced by new bone derived from the host. Carrying this line of thought to its logical conclusion he held that for clinical purposes it made no difference whether a bone graft was autologous, homologous or heterologous, or whether, at the time of transplantation, it was alive or dead. Subsequently, however, Barth (1908) abandoned this extreme view, and stated that living periosteum-covered bone was the best material for securing active new bone formation; he maintained, however, that for filling holes in the skull or in the long bones, dead bone chips or bone dust were equally satisfactory.

Axhausen (1909a) reported an extensive series of experiments in rats, rabbits and dogs, in which he used autotransplants, homotransplants and heterotransplants, of living and dead bone, with and without periosteum. His work differed from that of previous investigators in that he made much more detailed microscopical observations, but his conclusions were not very different from those of Ollier. He believed that in an osteo-periosteal transplant the bone proper necrosed, but much of the periosteum persisted and formed new bone to replace that which was destroyed. The periosteum functioned best when it was under a mechanical strain.

Meanwhile Macewen of Glasgow in 1881 had presented his celebrated paper on bone transplantation to the Royal Society of London, and had followed this by an extensive series of experimental and clinical observations which he gathered together in his treatise on the growth of bone published in 1912. Macewen, as we have seen, concluded that the periosteum was simply a limiting membrane and that all the phenomena of growth and repair in bone could be attributed to the activity of the osteoblasts, and he reported that orthotopic homografts of the whole or part of a long bone, even without their periosteum, were able to unite with host bone and to increase in thickness. This was confirmed in animals by McWil-

*'Les os dépouillés de leur périoste ne peuvent être greffés que chez les jeunes animaux (chien, lapin), et encore disparaissent-ils par résorption au bout d'un certain temps'

†The term orthotopic bone graft is used here to denote a graft which is placed in a skeletal defect and is in contact with host bone.

liams (1912 1914) Murphy (1912b) and *others* though McWilliams found that the proportion of surviving grafts was greater when the periosteum was retained

Baschkirzew and Petrow (1912) introduced a new hypothesis They maintained *that bone grafts became necrotic* but were subsequently regenerated through the activity of the surrounding granulation tissue which was stimulated to activity by the presence of the necrotic bone

Brown and Brown (1913) obtained results generally similar to those of Macewen but made the additional observation that heterotopic transplants and also orthotopic transplants which were not subjected to the stimulus of function atrophied and were absorbed thus conforming to Wolff's law according to which the size and form of a *bone are determined by the function which* it has to perform

Phemister (1914 1915) after numerous experiments with autotransplants in dogs concluded that the periosteum and endosteum played an important part in osteogenesis but that even when these structures were removed regeneration could still occur from surviving osteoblasts near the surface of the graft Bone chips regenerated more quickly than a single massive transplant and he attributed this to the fact that with chips there were more surviving osteogenic cells Phemister *emphasized the importance of* functional demand in stimulating regeneration and drew attention to the process of *creeping substitution* by which a bone graft may be gradually replaced by new bone without undergoing massive necrosis *Similar* conclusions were put forward subsequently by Smith (1916)

Gallie (1914 1919 1931) and Gallie and Robertson (1918 1919) also stressed the importance of surviving osteoblasts near the surface of a graft but unlike Phemister they concluded that the periosteum itself was non-osteogenic and did not facilitate regeneration Autogenous cancellous transplants were superior to cortical ones because they were revascularized more quickly and many more osteogenic cells survived

Davis and Hunnicutt (1915) also repeated and confirmed many of Macewen's experiments They found that periosteum either as a pedicle or a free transplant was non-osteogenic unless it had a thin layer of bone adhering to it

Groves (1917b) in his Jacksonian prize essay stated that the main facts concerning the biological aspects of bone grafting were already established and his own experiments in dogs and cats were designed primarily to determine the technical procedures most likely to be of clinical value He concluded that the ideal graft was a single piece of living bone used in its entire thickness and that fragments of living bone unless *closely in contact with vascular tissue* displayed no osteogenesis while dust formed from living bone did not maintain its vitality Cortical grafts he found to be far better than intramedullary ones which if small and loose neither assisted repair nor acted as a splint and if large enough to fit the marrow cavities of the fragments tightly hindered osteogenesis and were liable to fracture The success of a living graft he held depended very largely upon the extent of its contact with living bone the accuracy of its apposition and the firmness of its fixation Metal *sutures and pins he found useful in the fixation* of many grafts When properly applied they secured the necessary fixation and there was no evidence that they hindered osteogenesis Homografts under *favourable circumstances acted just as well* as autografts Dead bone grafts were certainly inferior to living when used to fill defects in the long bones but under favourable circumstances they became strongly in*corporated into the living skeleton* and for use as pegs or nails they appeared to be just as useful as living grafts Provided that secure fixation of the graft to its bed was not disturbed mobility of the limb favoured

osteogenesis, whilst immobilization hindered it

From 1919 to the Present Day

During this period the number of papers on experimental bone grafting has grown at a [illegible] rate. Despite the increasing use of [illegible]ogy and other special methods of investigation much of this work has been repe[illegible]and has either confirmed what was [illegible]dy known or has failed to resolve matters in dispute. Thus, for example, the phenomenon of creeping substitution in orthotopic transplants was described anew by Kornew (1929), Phemister (1930), Imbert (1933), May (1937) and others. The periosteum was reported to play an important role in the regeneration of bone transplants by Rohde (1925), Kartaschew (1930), Haldeman (1933), Keith (1934), and Vainio (1950), but this was denied by Ely (1922) and Pollock and Henderson (1940).

Some further advances have been made, however, and it will be convenient to consider these under the following headings.

1. Orthotopic autografts of living cancellous bone, callus and ground bone.
2. Orthotopic homografts of living bone.
3. Orthotopic heterografts of living bone.
4. Orthotopic grafts of dead bone.
5. Bone grafts in special sites.
6. Experiments using radioactive isotopes.

The development of methods of storing bone for grafting will be discussed in a later section.

Autografts of Living Cancellous Bone, Callus and Ground Bone. Bahls and Kalambokas (1937) studied the behaviour of autografts of cancellous bone from the ilium placed in defects in the femur in rabbits. They concluded that the grafts did not survive, but acted as a powerful stimulus of osteogenesis in the host bone. Murray, Holden and Roschlau (1957) found, however, that bone was also formed if the holes were filled with a plastic fenestrated cage. Other investigators, including Abbott, Schattstaedt, Saunders and Bost (1947) and Siffert (1955), have reported that grafts of cancellous bone regenerate rapidly from cells of the endosteum.

McKelvie and Mann (1948) studied cancellous bone grafts in rabbits with the object of elucidating the role of alkaline phosphatase in bone regeneration. They concluded that this enzyme was concerned more with the formation of bone matrix than with the deposition of calcium salts (cf. p. 355). Gomori (1952) produced conflicting results.

Klinkerfuss (1924) transplanted bone callus from healing fractures, and reported that this material survived and formed new bone more readily than cortical bone did.

Hoyer (1946), and Swanker and Winfield (1952), made defects in bones in rabbits and filled them with a paste made by grinding up fresh cortical and cancellous bone. They found that healing occurred more rapidly than when bone chips were used.

Homografts of Living Bone. Brooks and Hudson (1920), working with dogs, obtained complete regeneration of one of the long bones after resecting part of its shaft and replacing it with a homograft. Subsequent investigations in dogs and rabbits have confirmed this observation, but homografts have been found to be less reliable than autografts (Axhausen, 1953), and do not produce such rapid healing (Reynolds and Oliver, 1950). Moreover, as Hutchison (1952) and others have shown, homografts of bone do not survive but, if orthotopic, may be replaced gradually by bone of host origin.

Heterografts of Living Bone. Gallie and Robertson (1919) found that heterografts of dog bone in cats became encapsulated, and did not stimulate osteogenesis. Hughes (1943), on the other hand, found that heterografts of beef bone in dogs united with the

bone of the host, and appeared to facilitate the healing of bone defects

Grafts of Dead Bone. It has been shown in dogs, rabbits and other species that orthotopic grafts of autologous, homologous, and in some cases heterologous, bone which has been killed by boiling (Gallie and Robertson, 1919, Christophe, 1923, Davison and Christopher, 1921, Reynolds and Oliver, 1950), by storage in alcohol (Christophe, 1923), ether (Calvé, 1935) or merthiolate (Reynolds and Oliver, 1950), or by freeze drying (Kreuz, Hyatt, Turner and Bassett, 1951), stimulate osteogenesis and become incorporated in, and gradually replaced by, host bone The same has been reported to be true of bone which has been frozen and later thawed (Herbert and Paillot, 1950, Reynolds and Oliver, 1950, Marrangoni, 1951), but in the absence of special investigations such as tissue culture it is uncertain whether or not living cells were present in the grafts at the time of transplantation

Dead grafts appear to be less reliable than grafts of fresh bone, however, and several observers have reported complete failure (Imbert, 1925, Keith, 1934, Stewart, 1934) Moreover, even in the successful experiments, healing has usually been slower with dead grafts than with living ones (Reynolds and Oliver, 1950)

Materials known as *os purum* and *os novum*, originally prepared by Orell (1937) for clinical use, have also been used in experimental animals (Stark, 1942), and the results have been much the same as in humans These materials will be described later

Bone Grafts in Special Sites. Sandison (1928b) used his transparent chamber technique (p 31) to study osteogenesis in rabbits, and observed the formation of new bone from transplants of bone marrow with endosteum Subsequently Kiehn, Cebul, Berg Gutentag and Glover (1952), using a modified form of transparent chamber adapted for use in rats, observed that fresh autografts of bone were vascularized in 12 days, whereas autografts of boiled bone were treated as foreign bodies and became encapsulated

Hancox (1947) studied the behaviour of homografts of chick embryo bone on the chorioallantoic membrane Grafts of living bone became vascularized within 18 hours, apparently as a result of anastomoses between the capillaries of the host and the graft, whereas grafts of bone killed by boiling had not been revascularized by the end of 48 hours

Bone grafts in the anterior chamber of the eye have been studied by many observers (*see Lancet*, 1952) Urist and McLean (1951, 1952) found that grafts of periosteum were osteogenic if they were obtained from young rats, or from the vicinity of fractures in mature rats Osteogenesis was also observed after transplanting bone marrow or cancellous bone, but rarely after transplanting cortical bone It appeared that the new bone might be formed either from the graft, or from the host by a process of metaplasia, but it was not always possible to say which mechanism was involved Hutchison (1949, 1952) found in rabbits that autografts of bone with or without periosteum survived in the anterior chamber whereas homografts died and formed sequestra Ray, Degge, Gloyd and Mooney (1952) found however that homografts of embryo bone survived and grew

Kiehn and Gutentag (1955) studied the behaviour of bone homografts in diffusion chambers (p 114) which permit the passage of fluids and large molecules but not cells They found that such grafts were able to take up P^{32}, whereas homografts which were not isolated in this way failed to do so Subsequently, Duthie (1956, 1958) has made use of autografts of fracture callus, periosteum and cortical bone in diffusion chambers in an extensive histochemical study of osteogenesis

Further evidence that, in the absence of special protection, bone homografts are destroyed was obtained by Felts (1957), who transplanted the humerus from two day old mice subcutaneously to adult mice of the same, and also of a different, strain. He found that the isografts and homografts were indistinguishable for 12-16 days, but thereafter the homografts showed no further growth, their cells all disappeared, their marrow was replaced by connective tissue, and large necrotic areas appeared in their cartilage, whereas the isografts progressed to maturity

Experiments Using Radioactive Isotopes. Kiehn and his colleagues (Kiehn, Friedell and MacIntyre, 1948, Kiehn, Friedell, Benson, Berg and Glover, 1950, Kiehn, Cebul, Berg, Gutentag and Glover, 1952; Kiehn and Glover, 1953, Kiehn, Gutentag and Glover, 1954) have investigated the capacity of bone transplants in the subcutaneous tissue and in transparent chambers in dogs and rats to take up P^{32} following intravenous injection of dibasic sodium phosphate (P^{32}), and have taken this as an index of viability and also of vascularization The grafts were removed at intervals, and the radioactive count per gram of tissue per minute was determined with a Geiger counter after ashing the graft and dissolving the ash in nitric acid, or alternatively, histological sections were prepared and studied by autoradiography Dogs received 100-300 μc, and in rats it was found that 25 μc. gave the best autoradiographs.

It was found that fresh autografts took up P^{32} promptly, the uptake per gram of tissue in 24 hours being 60 per cent of that of normal bone Autografts which had been stored at 0°C * took up P^{32} more slowly than fresh grafts for the first two or three days, but at the same rate thereafter. Kiehn *et al* (1950) interpreted this as evidence that the stored grafts were able to metabolize phosphorus like fresh grafts, but Key (1950) has suggested that even a non-viable graft might absorb P^{32} to the extent observed. Boiled autografts, fresh and boiled homografts, and homografts which had been stored at 0°C. for a month or longer, however, showed a persistently low uptake, and this would seem to reflect some fundamental difference in their biological status.

Clinical Observations

Bone grafting as a clinical procedure became possible with the development of anaesthesia and asepsis. The pioneer in this

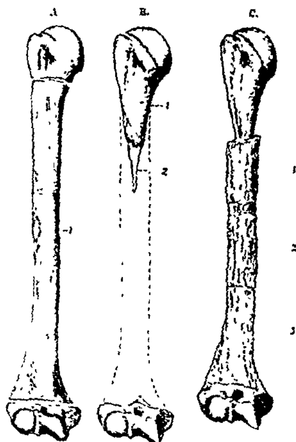


Fig 114 Macewen's illustration of the first successful bone homograft in a human A. 1 Necrosed diaphysis which was removed. B. 1. Portion of shaft attached to head reproduced from original periosteum 2 Cartilaginous terminal removed before first transplant C 1 First graft 2. Second graft 3 Third graft (Reproduced from *Proceedings of the Royal Society* by courtesy of the Royal Society)

*Kiehn *et al* refer to these grafts as frozen but 0°C is clearly above the freezing point of the intracellular and extracellular fluid

field was Macewen of Glasgow, who performed the first successful *homograft* of bone in a human in 1878 (Fig. 114). The recipient was a child with osteomyelitis in whom the entire shaft of the humerus had sequestrated and failed to regenerate. *Instead of amputating the arm* Macewen transplanted on three occasions bone chips from pieces of tibia obtained from three other patients while performing wedge osteotomy for rachitic deformity. The humerus was reconstituted and a useful arm resulted. Macewen subsequently used homografts in many other cases, and he gave a full account of some of these in his celebrated book on the growth of bone (Macewen, 1912).

Previously, Nussbaum (1875) had rotated a fragment of ulna to bridge a two inch defect in this bone, thus performing the first recorded human bone autograft, but apart from this earlier attempts at bone grafting were curiosities, and usually involved animal bone. This is exemplified by the "fabulous" case, reported by a clergyman during the 17th century and subsequently cited by Macewen (1881), in which a defect in a soldier's skull was replaced by a piece of the skull of a dog, hair and all. It is recorded that the heavens opened, thunder and lightning rent the air, and nearly struck the soldier. This was taken as a sign that the graft was unclean, and the man was called *to account for it! According to Henderson* (1914a, b) the graft took successfully, but the ecclesiastical authorities insisted on its removal. Macewen himself also filled a skull defect in a child with a graft from the skull of a young dog but the result was equivocal.

Following Nussbaum further cases of autografting were reported by Curtis (1893), who among other things used a piece of fibula to repair an ununited fracture of the tibia; Bardenheuer (1896), who replaced the shaft of the humerus, which had sequestrated as a result of osteomyelitis, with part of the spine of the scapula; Murphy (1912b), who tried unsuccessfully to repair a nose using a phalanx from a supernumerary digit, and others, but the procedure was not widely used until the early years of this century when autografts of cortical bone began to be used in place of the metal plates introduced by Lane (1913, 1914) for the treatment of fractures, and which, owing to the nature of the metal *and the high incidence* of infection, had produced such disastrous results.

As the scope of orthopaedic surgery increased, and the dangers of operation diminished, the use of cortical autografts was extended to include not only the treatment of both recent and *ununited* fractures, but also the repair of defects in bones occasioned by injury, disease or surgical operations, and the arthrodesis of joints, including those of the spine. A landmark was reached in 1915 when Albee published his celebrated book on bone graft surgery, in which the indications for bone grafting* were fully set out, together with a detailed account of the technique of *cortical* inlay grafting which Albee developed and popularized, and a description of the special instruments which he devised including his electrical saw.

The cortical inlay graft is illustrated in Figure 115. A second type of graft, the massive onlay graft (Fig. 116), was developed by Henderson of the Mayo Clinic (Henderson, 1914a, b, 1920, 1921, 1923, 1928, 1936, 1938). Yet a third type of cortical bone graft, the intramedullary peg, was used by Hoglund (1917), and received favourable comment from Groves (1921), but proved to be unsatisfactory and is no longer used except occasionally in a limited form as for example in pegging fractures of the medial malleolus of the ankle.

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*Albee's indications for bone grafting were rather more numerous than would be admitted today. He used the procedure in the treatment of many recent fractures (including fractures of the neck of the femur) and ununited fractures, for spinal fusion, for arthrodesis of the sacro-iliac, knee and ankle joints, for stabilization of the foot, and for repairing defects in the skull and long bones.

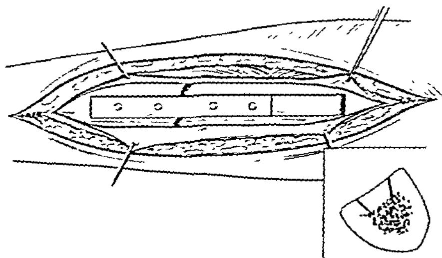


Fig. 115. Cortical inlay graft. The graft is cut with an electric saw from the proximal fragment placed across the site of the fracture, and fixed with vitallium screws.

It gradually became apparent that purely cortical bone was not the ideal form of graft (see *British Medical Journal*, 1957). Albee himself advised including a certain amount of what he called endosteum (meaning by this cancellous bone) with grafts cut from the tibia, and this practice was followed by many surgeons including Phemister (1914), Campbell (1925) and Gallie (1931). Others, such as Carter (1911, 1930) and McWilliams (1912) used segments of rib for reconstruction of the nose and mandible respectively, and thus achieved a mixture of cortical and cancellous bone in another way.

The clinical observations of Todd (1920) on the role of cancellous bone in the healing of chronic osteomyelitis, and the experimental findings of Berg and Thalhimer (1918), Matti (1931, 1932), Ghormley and Stuck (1934), and others, encouraged the further step of using grafts of purely cancellous bone. Matti himself in 1926 used iliac cancellous bone in a patient with an ununited fracture of the radius but the operation was unsuccessful. Later, however, he was able to report a large series of cases in which he had successfully used this material (Matti, 1932, 1936). Subsequently, autografts of

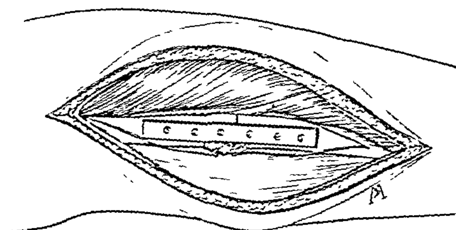


Fig. 116. Massive onlay graft. The graft is secured with vitallium screws which reach the cortex on the opposite side of the shaft. Cancellous bone is also packed around the site of the fracture.

cancellous bone, and especially of iliac bone, have been used successfully by many surgeons for a variety of purposes including the treatment of ununited fractures (King 1948 Dick 1946 Higgs 1946) the repair of defects of the jaws (Mowlem 1944, Blocker and Weiss 1946), skull (Mowlem 1944 Dick 1946) and long bones (Dick, 1946 Robertson and Barron 1946) reconstruction of the nose (Gillies 1923 Mowlem 1941, 1944, 1945 Horowitz 1949), fusion of the spine and other forms of arthrodesis (Dick 1946 Abbott, Schottstaedt Saunders and Bost 1947)

Gelatinized bone made by grinding iliac cancellous bone with fibrin foam and thrombin to form a cohesive putty like mass was introduced by Sheehan and Swanker (1950) who used it for a variety of purposes including filling defects in the mandible and floor of the orbit and levelling out depressions of the nasal bones malar bone maxilla and cranium

Following Macewen fresh homografts of bone obtained at operations or from cadavers have been used by surgeons in various parts of the world including Poncet (1887) Budinger (1900), Kuettner (1911 1913) Ullmer and Schmincke (1925) Smith (1937) Ghormley (1942) Henry (1948), and Harbin and Eiber (1949)

The articular ends of bones and even whole joints have also been used as grafts but this work will be considered in conjunction with the transplantation of cartilage (Chapter 19)

Neuhof and Hirshfeld (1923) cited a number of cases in which homografts of foetal bone were used but no convincing evidence has been brought forward to suggest that this material is better than adult bone

As might be expected there has been much difference of opinion regarding the fate of bone homografts in man The view expressed by Borst in 1948 that such grafts act simply as a scaffolding and are gradually replaced by bone of host origin has steadily gained ground and is now generally accepted (*see* p 357) though as recently as 1949 Harbin and Eiber expressed the view that a large proportion of fresh homografts survive

With the development of bone banks the use of fresh bone homografts has greatly diminished The methods of storage which are used and the history of their development will be considered in the next section

Very few cases have been reported in which heterografts of fresh living bone have been used Heterografts of stored bone will be considered in the next section

STORAGE AND STERILIZATION OF BONE GRAFTS

The general principles governing the storage and sterilization of tissues for grafting have been considered in Chapter 9 Here we shall consider the use of the following methods for the storage and sterilization of bone

1 Storage at ice box temperature
2 *Freezing*
3 Storage at room temperature after *freeze drying*
4 Storage and sterilization in antiseptic solutions
5 Sterilization by boiling
6 The preparation of *os purum* and *os novum*

Storage at Ice Box Temperature

Albee (1915) stored human bone in sterile petrolatum at a temperature of between 1 and 5 C He used this material in a few cases and claimed some good results

The method was revived many years later by Inclan (1942) who stored human bone for periods ranging from three to sixty three days at 2 to 5 C in citrated blood from the donor or another person or in a few instances in saline or saline mixed with a small amount of blood Inclan used autografts and also homografts of both adult

and foetal bone. He recommended storing autografts in this way when a two-stage operation was required, as for example when performing extensive spinal fusion in children. He used homografts when it was impossible to obtain bone from the patient himself, and as a rule he obtained them from a voluntary donor. Though he preferred to use fresh autografts whenever possible, he concluded that refrigerated autografts and homografts behaved in much the same way.

Bush (1947) stored bone from amputated limbs, and also from cadavers, in sterile jars at temperatures between 2° and 5°C for periods up to three weeks He used homografts of this material in 67 cases, mainly for spinal fusion operations, and found it very satisfactory

Refrigeration short of freezing has also been used in conjunction with storage in antiseptic solutions (*v infra*)

Freezing

P D Wilson (1947), impressed by the fact that to obtain an autograft meant prolonging the operation and left a bony defect, began to collect healthy bone removed in the course of orthopaedic operations, and to store it in sealed jars in a deep freeze unit at between −10° and −20°F. Care was taken to see that the donor was free from syphilis, malaria, hepatitis or other recent infection, and a sample of the bone was cultured at the time of bottling The donor's blood group was ignored. In his initial report Wilson stated that he had used bone from 40 donors at 30 operations, the period of preservation ranging from one to 89 days. In every case the wounds healed by first intention and remained healed, and in no case did the graft slough. Cavities and bone defects became obliterated and fractures united, and in all cases the clinical behaviour of the grafts seemed to be identical with that of fresh autologous bone Histologically too no difference was observed between fresh autologous and stored homologous bone, except perhaps for the clinically unimportant survival of a few cells in fresh autografts. Wilson was thus encouraged to expand this work, and to advocate the establishment of bone banks for storing frozen bone obtained not only from surgical operations, but also from cadavers. In later papers (1951a, b) he reported 85 per cent successful results in 218 patients who received homografts of frozen bone. He found that grafts preserved for periods of a year were satisfactory, but beyond this the transplants deteriorated and the failure rate increased sharply.

Bush and Garber (1948) used homografts of frozen bone in the form of bone chips in 43 patients, mainly in spinal fusion operations for scoliosis In their view the grafts were dead, but despite this the results were very satisfactory. Weaver (1949) also found that chip grafts of frozen bone gave excellent results, but massive grafts of frozen cortical bone used in the treatment of ununited fractures, though sometimes highly successful, were on average distinctly inferior to autografts

Lipscomb (1949) concluded that frozen bone should not be used to the exclusion of fresh autografts, but was valuable (a) in children, where the supply of autologous bone was often insufficient, (b) for filling large cystic cavities and defects resulting from removal of giant cell tumours, and (c) for reinforcing autografts.

Converse and Campbell (1950) stored bone at −50°C. for periods up to 436 days, and used both autografts and homografts of this material in treating depressions in the skull, saddle-nose deformity, and defects in the mandible Both homografts and autografts gave excellent results.

Homografts of frozen bone have since been used by many surgeons including Stuck and Dandridge (1950), Sicard and Binet (1950), Harmon (1950), Zimbron

(1950), Herbert (1951), Capurro and Pedemonte (1953), and Abbott (1953)

Judet and Arviset (1919) used heterografts of frozen calf bone for a variety of purposes including the treatment of both recent and ununited fractures, spinal fusion, and the treatment of bone cysts. The results were reported to be encouraging, and subsequently heterografts of frozen bone were used by a number of other French surgeons including Guillemmet, Stagnara and Dubost Perret (1950, 1953), and Judet and Judet (1953) Judet and Judet used pig, calf and colt bone They concluded that frozen heterografts were just as good as homografts obtained either at operation or from cadavers

Experiments in animals, though on a less extensive scale, have confirmed the clinical observation that bone stored at −20°C. or lower and subsequently transplanted orthotopically is capable of being systematically replaced by host bone In rabbits Bush and Garber (1918) found that autografts and homografts which had been stored frozen for two weeks or less behaved similarly, and even after longer periods of storage, autografts were replaced only slightly more quickly than homografts, while Turner (1952) showed that frozen autografts were replaced as completely as fresh autografts and almost as quickly

There appears therefore to be little incentive to change the technique of storage, although on theoretical grounds (Chapter 9) one might expect storage at −70°C or lower to give better results than storage at −20°C, especially if the bone were soaked in glycerol saline before being frozen

Freeze-Drying

Homografts of freeze-dried bone have been used extensively by Hyatt and his colleagues (Hyatt, 1950, Kreuz, Hyatt, Turner and Bassett, 1951, Hyatt and Butler, 1957) at the United States Naval Medical Center, Bethesda Generally speaking the bone, obtained from cadavers, has been minced before being freeze-dried, and this material has been reported to give excellent results

As we have seen (Chapter 9) freeze drying does not necessarily destroy bacteria, and therefore the bone must either be obtained with full aseptic precautions, or must be sterilized in some way Bassett, Hudgins, Trump and Wright (1956) used cathode rays generated at high voltage for this purpose, and DeVries, Kempe and Brinker (1955) have used gamma rays from radioactive cobalt The value of these procedures must be regarded as still *sub judice*

Storage and Sterilization in Antiseptic Solutions

Christophe (1923) used homografts of bone which had been stored in alcohol In one patient he used a homograft of a patella with the ligamentum patellae and the quadriceps tendon He reported that the wound healed by first intention and that the patient, who was a soldier, returned to duty four weeks later, but it is somewhat difficult to credit this He also reported good results in two patients who received grafts of alcohol preserved bone to defects in the radius and ulna respectively

Reynolds and Oliver (1949) stored bone obtained at operation, and also from cadavers, in merthiolate solution The periosteum was removed and a sample of bone marrow was taken for bacteriological culture The bone was then put in jars containing a 1 1000 aqueous solution of merthiolate After two weeks the grafts were transferred aseptically to fresh jars containing a 1 5000 solution of merthiolate, and further samples were taken for bacteriological culture Thereafter the solution was changed every two weeks, and further cultures were taken The bone was never used until at least two cultures had been reported as negative The jars were kept either at room temperature or in an ordinary refrig-

erator. Before use the grafts were rinsed in sterile normal saline. Reynolds and Oliver used homografts of merthiolate-preserved bone in 42 patients for a variety of purposes including spinal fusion (15 cases), the treatment of ununited fractures (9 cases), the obliteration of defects in bones occasioned by disease or operation (10 cases), the treatment of recent fractures (2 cases), and the treatment of aseptic necrosis of the femoral head (1 case). The early phase of healing progressed more slowly than with fresh autologous bone and a more prolonged period of immobilization was therefore required, but the final result was good in 35 of the 42 cases.

Later, in the light of experience of merthiolate-preserved bone in 212 operations, Reynolds, Oliver and Ramsey (1951) concluded that this material is a useful adjunct to, but not a universal substitute for, fresh autologous bone. This is borne out by the experience of many surgeons in various parts of the world including Harmon (1950), Gordon and Welsh (1951), and Nisbet and Ellis (unpublished).

Calvé (1935) used heterografts of cancellous calf bone which had been stored in ether for spinal fusion, and also in arthrodesing other joints, and reported excellent results.

Sterilization by Boiling

Kausch (1906, 1909a, 1910) replaced the upper end of the tibia after resecting a sarcoma with a homograft of bone which had been obtained from an amputated limb and subsequently treated with alcohol and ether and then boiled. Amputation had to be performed nine months later for recurrence of the tumour, and at that time there appeared to be perfect union between the graft and the host bone. A somewhat similar case was reported by Leriche (1913), who used boiled cadaver bone after resecting a sarcoma of the upper end of the humerus. Once again the graft united, but the tumour recurred a few months later and a forequarter amputation had to be performed.

Gallie and Robertson (1918) used boiled bone in 60 operations, and reported that the graft united in every case and gave an excellent clinical result.

More recently Lloyd-Roberts (1952) reported a series of 34 cases in which he used homografts of boiled bone, mainly for the arthrodesis of joints. In 26 patients the operation was regarded as successful. In the following year Rocher (1953) reported over 200 cases in which homografts of boiled cortical or cancellous bone were used. The results on the whole were good, but Rocher emphasized that it was preferable to use fresh autografts for spinal fusion and for bridging gaps in long bones.

Gallie and Robertson (1919), and Henderson (1920), used plates and screws of boiled beef bone extensively in the treatment of fractures. Their example was followed by many other surgeons, but nowadays it is more usual to use screws made of an inert metal.

Os Purum, Os Novum and Anorganic Bone

Orell (1937) of Stockholm freed bone of fat, connective tissue and protein by "a lengthy physico-chemical process" which included soaking in warm potassium hydroxide, and he called the resulting material *os purum*. He reported using this material in 50 patients, mostly following resection of bone for tuberculosis. The results were good, except when abscesses or fistulae were present in which event the graft was often extruded. Complete replacement of the graft by new bone often took between two and three years.

In other patients Orell buried a suitably shaped piece of os purum sub-periosteally over the anteromedial surface of the tibia. One to two months later the graft was excised, and was found to be surrounded and

permeated by a profuse growth of new soft vascular bone. The graft transformed in this way was termed *os purum* and was used successfully by Orell for spinal fusion and in the treatment of ununited fractures.

Os purum prepared according to Orell's method has since been used by a number of surgeons including van der Hoff (1944) and Goff (1944). This material has not been successful in the treatment of ununited fractures or in the arthrodesis of joints and is used mainly to obliterate cavities. Somewhat similar material prepared by a rather more complicated process has been used recently by Maatz and Bauermeister (1957) in 200 patients with excellent results.

Hurley (1957) and Boyne and Losee (1958) attempted to remove the organic part of bone more or less completely while leaving the inorganic part unchanged. The material prepared in this way, which they called *anorganic bone*, has been used with success both experimentally and clinically for filling cavities in bone and building up the facial and alveolar contours.

CURRENT VIEWS ON THE FATE OF BONE GRAFTS

Despite the various conflicting opinions which have been expressed, it appears almost certain that massive orthotopic autografts of cortical bone are to a large extent replaced by host* bone.

The histological evidence suggests that the process of replacement proceeds in an irregular fashion, so that in some areas the cells of the graft seem to be alive while in other areas the lacunae are either empty or the cells take up the stain poorly (Imbert 1930; Ghormley and Stuck 1934). The union of cortical bone grafts is therefore slow (Ghormley 1942). It seems clear however that such a graft is not merely a source of minerals as Leriche and Policard (1928) suggested, but both stimulates and controls the process of regeneration. It is customary to say that the graft acts as a scaffolding, but in using this metaphor we must beware of thinking that the graft subserves only some purely mechanical function.

On the other hand it seems equally certain from clinical and experimental evidence that small autografts of cancellous bone behave quite differently (Mair 1932; Mowlem 1944). It is known that under certain conditions such grafts may survive for a long time even in heterotopic sites, and there is no reason to doubt that orthotopic grafts can do likewise.

Grafts of dead bone, whether autologous, homologous or heterologous, obviously cannot be said to survive. They too, however, appear to stimulate, and to some extent to control, bone regeneration, but the process of repair is significantly slower than with autografts of living bone.

Interest therefore centres on the immunological properties, and consequential behaviour, of fresh homografts and heterografts, and on the extent to which these immunological properties are lost or modified by procedures such as freeze-drying which render the graft non-viable. There is no reason to suppose that bone grafts differ significantly in their immunological properties from corresponding grafts of other vascular tissues, and the observations of Bonfiglio, Jeter and Smith (1954), Curtiss and his colleagues (Curtiss and Herndon 1956; Curtiss, Chase and Herndon 1956) and Enneking (1957) in rabbits, dogs and rats respectively suggest that this is in fact the case, and that homografts of living bone are effectively antigenic. Comparatively little work has been done in this field, however, and the matter clearly merits further investigation.

*The meaning attached to this term in relation to a graft is explained on p. 4

BONE GRAFTING IN PRESENT DAY SURGERY

Types of Graft Used

Autografts

Sliding inlay grafts of cortical bone are still used extensively in the treatment of fractures of the tibia showing delayed union or *non-union*, but for most purposes cancellous grafts are used, either alone or in conjunction with cortical grafts In the treatment of ununited fractures cancellous onlay grafts are often used in conjunction with some mechanical form of internal fixation.

Cortical grafts are commonly obtained from the tibia (*see* Fig. 121), and cancellous grafts from the ilium (*see* Fig. 120). Ribs provide a convenient source of both cancellous and cortical bone, and are especially useful for filling defects in the skull.

As a general rule autografts of fresh living bone are used, but occasionally if an autograft is accidentally contaminated, for example by being dropped, it is boiled and then used Cases have also been reported in which part of the mandible has been resected for neoplastic disease, and subsequently boiled and replaced (Harding, 1957)

Homografts

For homografts stored bone is generally employed The methods of storage most commonly used are preservation in merthiolate solution and freezing. Frozen bone is usually stored in commercial deep freeze at −20°C., but as we have seen (p. 176) it might be advantageous to store it at −70°C or lower Freeze-drying requires more elaborate equipment, and perhaps for this reason has not proved so popular. Freeze-dried ground cancellous bone is prepared on a large scale however by Hyatt and his colleagues at the tissue bank operated by the United States Navy, and distributed to hospitals in America and elsewhere. Homografts of this material appear to be at least as good as homografts of bone stored in other ways.

A considerable amount of bone for storage may be obtained at operation, notably ribs removed in the course of thoracoplasty, but large bone banks must rely on cadaver bone for their main source of supply. This is normally obtained with full aseptic precautions even when the merthiolate method of storage is to be used. It seems likely, however, that as methods of sterilization by irradiation are improved this rather tedious procedure will no longer be necessary.

Heterografts

Stored heterografts are still used in some hospitals, but should now be regarded as obsolete.

Indications for Bone Grafting

The indications for bone grafting will be discussed under the following headings:

1. The treatment of recent fractures.
2. The treatment of fractures showing delayed union and non-union.
3. Bridging defects in bone.
4. Restoration of contour.
5. Arthrodesis.
6. The replacement of digits.

It must be emphasized that the order is one of convenience, and does not reflect the relative importance of bone grafting in the conditions mentioned.

Recent Fractures

Occasionally, as Verbrugge (1946) and others have advocated, cancellous bone grafts may be used in conjunction with some form of internal fixation in the treatment of a recent fracture, but as a rule it is better to rely on simpler methods. Indeed the need for bone grafting fractures would probably be less if open reduction of fractures were resorted to less frequently. As long ago as 1911 Albee said that the results of open reduction were superior only to the results of bad conservative treatment, and

while this may be an exaggeration it is probably nearer to the truth than the view expressed in more recent times by Kennedy (1911) and others that early open reduction is to be desired in practically all fractures Robert Jones (1916b) said that neither plating nor bony fixation seems necessary in any other ordinary fracture of the limbs The exception to which he was referring was the oblique unstable fracture of the distal third of the tibia for which he advocated *immediate open reduction* and a sliding inlay graft used not to stimulate osteogenesis but in lieu of a metal plate to prevent rotation Today vitallium screws or a plate would be used instead *Watson Jones* (1952) blames the surgeon for the non union of fractures and not the osteoblast

Delayed Union and Non union of Fractures

There is no sharp distinction between delayed union and non union but as a general rule when the term *non union* is used it means that bone grafting is necessary to achieve union At an earlier stage bone grafting may also be used but simpler methods *may* suffice and as Robert Jones (1913) said many years ago non union would rarely occur if delayed union received proper attention

Bone grafts are used in these *conditions* primarily as osteogenic stimulants They may subserve a subsidiary function as internal splints but some other form of immobilization is essential in addition

Cortical grafts of either the onlay or inlay type are still used often in combination with grafts of cancellous bone (Figs 117 118) Many surgeons however are dispensing with cortical grafts and using only strips of cancellous bone obtained from the ilium (see *British Medical Journal* 1957) If the fragments are not unduly displaced it is sufficient to freshen the cortex above and below the fracture line and surround the shaft subperiosteally at the level of the fracture with the cancellous strips in much the same way as the cortical *onlay graft* advocated by Phemister (1931 1947) External splinting with a plaster cast is essential *Displaced* fragments must be reduced and placed end to end before the grafts are applied and internal fixation with a stainless steel or vitallium plate or with an intramedullary nail is required as well as a plaster cast

Autografts give the best results *Homografts* are also effective however though healing is not as rapid as with autografts and are used by some surgeons in order to avoid the additional trauma of obtaining autologous bone

Bridging Defects in Bone

*Gaps in long bones resulting from gun*shot wounds and other compound injuries require bone grafting but this must be postponed until infection has subsided and adequate skin cover has been obtained This may entail a delay of many months and in consequence there is often gross scarring and the failure rate is high but several series of successful cases have been reported (Jones 1916 Mitchell 1922 Key 1943 Thibodeau 1944 Lipscomb and Evans 1949)

It is essential to use autologous bone Most surgeons use either one or two cortical onlay grafts in conjunction with grafts of cancellous bone In the special instance of congenital pseudarthrosis of the tibia this method has been of great value (Boyd 1941 1943 Boyd and Fox 1948) Recently however Nicoll (1956) has obtained good results in bridging long defects with autografts of fresh cancellous bone combined with both internal and external *immobilization*

Bone grafts have long been used to bridge gaps in the mandible resulting from injury infection or neoplastic disease Macewen (1912) successfully used a graft *of rib for* this purpose and much more recently Converse (1950) has used stored bone but most

surgeons prefer to use a graft of fresh autologous bone from the crest of the ilium Scar tissue must first be excised, and full thickness skin cover achieved, if necessary by means of a pedicle graft. All sepsis in the mouth must be eliminated. Generally speaking the area is then exposed externally, care being taken not to open the buccal mucous membrane, and the graft is firmly wired to the fragments Various forms of dental splinting may be required until union is firm. After resecting half the mandible it may be replaced immediately by a graft, or alternatively an acrylic prosthesis may be inserted and later, if it seems necessary, replaced by a graft.

Bone grafts have also long been used to repair defects in the cranium (Fig. 119), and autografts of rib have proved to be particularly suitable (Longacre and Destefano, 1957). In recent years, however, there has been an increasing tendency to use acrylic



Fig 117 Radiograph illustrating successful treatment of an ununited fracture of the tibia of 6 months' duration by onlay grafts of cancellous bone from the iliac crest held in place by suture of the muscles.

Fig 118 Radiograph illustrating successful treatment of an ununited fracture of the femur of 9 months' duration treated by freshening the bone ends, inserting an intramedullary nail and applying onlay grafts of cortical bone. The grafts were held in place by suture of the muscles.

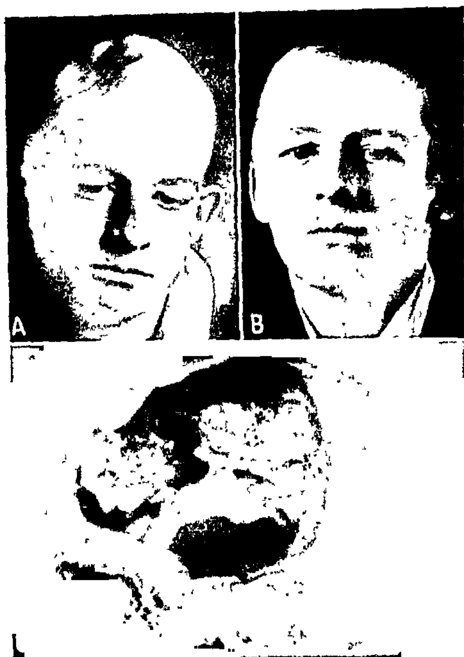


Fig 110 Large cranial defect due to osteomyelitis repaired by a graft of os purum prepared from the skull of a cadaver. A, B. The patient before and after operation. C Radiograph showing that much of the graft has been absorbed but enough remains to afford adequate protection (Mr Murray Falconer's case)

prostheses instead of bone grafts for this purpose (Robinson and Macalister, 1951).

Restoration of Contour

Grafts of iliac cancellous bone, rib bone, and in appropriate cases bone from the nasal septum, have been used extensively in the treatment of saddle nose and to repair other unsightly depressions in the head and neck region. Alternatively cartilage is used (Chapter 19), but many surgeons, including Mowlem (1941) and Peer (1951), prefer bone on the ground that the results are more permanent. Converse (1951) reported that fresh autologous bone gave the best results, and this has certainly been the material most widely used, but good results have also been obtained with homografts (Peer, 1951).

Arthrodesis

Bone grafts have been used extensively in the operation of spinal fusion, and in arthrodesis of the hip and other joints.

In Hibbs' (1911, 1912) operation for spinal fusion, which is used extensively in cases of tuberculosis of the spine, spondylarthrosis and paralytic spinal deformities, the intervertebral joints are excised, and bone grafts in the form of slivers of the spinous processes are turned upwards and downwards to overlap each other. Albee's (1911, 1913, 1914) operation, in which the spinous processes are split and bridged by the insertion of a massive cortical tibial graft, had a tremendous vogue for the treatment of tuberculosis of the spine, but doubt was thrown on the efficacy of this operation by Gallie as long ago as 1931 and it is now almost obsolete for this purpose. Instead, twin cortical grafts are commonly laid along the roughened spines and laminae, as advocated by Girdlestone (1931, 1932). Another effective procedure is that introduced by Bosworth (1942) in which a clothes peg type of massive iliac bone graft is used as a bridge between two spinous processes, usually in the lower lumbar region. Attempts have also been made with some success to fuse adjacent vertebral bodies (Cloward, 1953a, b; James and Nisbet, 1953) but the technical difficulties of these operations have limited their use. Gross paralytic spinal deformities may require extensive bone grafting in a series of staged operations after the deformity has been corrected by some form of external splinting (Risser, 1956). Homografts of banked bone are often used in order to reduce the magnitude of the operation.

Arthrodesis of other joints is performed for a variety of reasons, including relief of pain in chronic arthritis associated with unsound ankylosis, and the treatment of tuberculous arthritis. It was thought for many years that the best result which could be obtained for a tuberculous joint was sound bony ankylosis. With improved public health measures and antibiotic therapy, however, the prognosis in this condition has greatly improved, and arthrodesis is now much less used.

Joints may be arthrodesed by intra-articular or extra-articular procedures, or by a combination of the two. In intra-articular arthrodesis the joint surfaces are removed until fresh bone is exposed, and the space between the bone ends may be filled with cancellous bone or bridged with a cortical bone graft (*see* Fig. 125). In extra-articular arthrodesis the joint is not opened, but a graft is used as a bridge between the bones taking part in the articulation (*see* Fig. 124). Thus, for example, extra-articular arthrodesis of the hip may be performed by inserting a massive tibial cortical bone graft between the great trochanter and the ilium (Albee, 1939), by turning up a graft from the great trochanter and embedding it into the ilium (Hibbs, 1926; Sorrell, 1930), or by inserting a graft between the ischial tuberosity and the upper part of the shaft of the femur (Trumble, 1932; Brittain, 1948).

Whatever form of arthrodesis is used it is of course essential to fix the joint in the optimal position for function. The proper position for each joint, and many other matters relating to the performance of arthrodesis, are dealt with by Steindler (1940) and Campbell (1949). A good historical account of the development of the methods of arthrodesis is given by Bick (1948).

Replacement of Digits

Bone grafting plays an important part in the reconstruction of digits (Gordon, 1944; Blake, 1948; Gillies and Reid, 1955). Many ingenious methods have been devised to reconstruct the thumb, including transplantation of a toe (Rank and Wakefield, 1953).

Technical Considerations

Patients who are admitted to hospital for a bone grafting operation must be in good health and free from infection of the skin.

The skin is shaved the evening before operation* and the patient has a bath. In the morning the skin is painted with cetrimide, followed by spirit, and the part is wrapped in sterile towels. It is sometimes possible to apply splintage beforehand to minimize the operative trauma, for example before extensive fusions of the spine, and it may be necessary to arrange for a blood transfusion. A tourniquet is usually applied to a limb which is to receive a graft, or from which a tibial bone graft is to be removed. The grafts, either from the tibia or ilium, are best obtained simultaneously by a separate team of surgeons. Once the skin incision has been made, the skin edges are draped with towels and it is customary to use what is called a "no touch" technique, which means that all tissues including the grafts are handled as far as possible only with instruments. Complicated bone grafting operations should be reserved for centres which specialize in them.

Source of Autologous Bone Grafts

To obtain cancellous bone grafts (Fig. 120) the iliac crest is exposed through a curved incision near the anterior superior spine or, if the patient is in the prone position for a spinal fusion, from the region of the posterior superior spine. The attachments of the abdominal and pelvic muscles are carefully stripped and the periosteum is removed from the cortex. Strips of bone are cut with an osteotome and extra cancellous bone may be obtained with a sharp spoon. The abdominal and pelvic muscles are closely sutured to obliterate the dead space, to secure haemostasis and prevent incisional hernia.

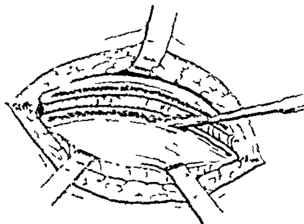


Fig. 120. Method of obtaining thin grafts of cancellous bone. The ilium is exposed on both aspects and the grafts are cut with an osteotome. The iliac crest may be retained as shown and sutured back in place. The muscles are sutured closely to obliterate the dead space and avoid subsequent herniation.

*Shaving the day before operation is omitted in some clinics because it is believed that scratches so caused may harbour staphylococci. The part may be shaved on the operating table if necessary.

Cortical bone grafts are obtained from the tibia as described below.

Treatment of Ununited Fractures of the Shaft Bones by the Massive Inlay Graft

Fractures of the tibia at the junction of the middle and lower thirds quite commonly fail to unite, and are thus suitable for a sliding massive cortical inlay graft of the Albee type, obtained from the proximal fragment (*see* Fig. 115). The bone is exposed through a longitudinal incision and the periosteum is stripped. The graft is cut with an electric saw, the blade being kept cool by a continuous drip of cold saline. The saw cuts are made just within the anteromedial and posteromedial borders of the subcutaneous surface of the tibia, and the saw blade is held obliquely so that the cut reaches the medullary cavity and does not become implicated in the dense cortical bone at the angles. This also ensures that the graft is easily elevated from its bed and does not disappear into the medullary cavity. The saw cuts are continued across the site of the fracture and into the distal fragment, the distal part of the graft is removed, and the large proximal part is then lifted out of its bed and replaced immediately between the two fragments. It is held in place with vitallium screws, two in each fragment if possible. This dispenses with the need to obtain the perfect fit required in the classical Albee technique. The piece of

bone from the distal fragment is replaced in the gap in the proximal fragment. As no new tissue has been introduced it is often possible to suture the periosteum as well as the skin. A plaster cast is applied until union is sound.

A similar operation is sometimes used for arthrodesis of the ankle.

A massive cortical graft for use in another site is obtained in the same way (Fig 121). The cancellous bone on the medullary surface is retained.

All metal screws and plates which are buried in association with bone grafts must be made from a strong inert alloy, such as vitallium or 18-8 S. Mo stainless steel The latter is also known as Enduro stainless steel and is an alloy of steel and molybdenum.

Treatment of Ununited Fractures of the Shaft Bones by the Massive Onlay Graft

The shaft and site of the fracture are exposed subperiosteally, usually using one of the exposures described by Henry (1946), and one side of the cortex is flattened with an osteotome to receive the graft, care being taken not to enter the medullary cavity The massive cortical graft procured from the tibia is laid with the endosteal side against the fragments, and centred on the site of fracture and held in place with vitallium screws, at least two in each fragment. It is essential for the screws to reach the opposite cortex. Cancellous bone obtained from the bed of the graft in the tibia is packed around the site of fracture. External splintage in the form of a plaster cast is required until union is sound.

Bridging Gaps in Shaft Fractures

The soft parts often require prolonged attention in order to clean up infection, and to replace skin loss tubed pedicle or cross leg flaps may be required.

The method described by Nicoll (1956) has given good results, especially in the upper limb. The fracture site is widely exposed, the fragments are re-aligned and the sclerosed bone ends are excised. A block of cancellous bone is cut from the ilium and inserted between the bone ends. A metal plate is then placed as a bridge between the fragments and held with screws. Prolonged splinting in a plaster cast is then required.

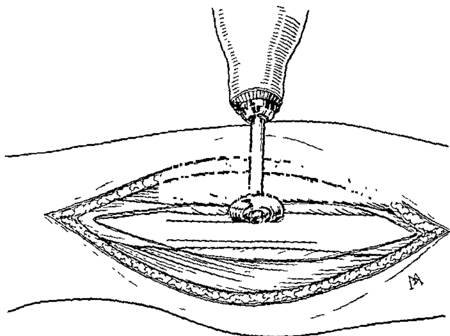


Fig. 121. Method of cutting a massive tibial bone graft The tibia is exposed subperiosteally and the graft is cut with an electric saw.

Alternatively double onlay grafts may be used (v infra) and are more suitable in the lower limb

In adults gaps in the tibia resulting from fractures may also be bridged by transference of the fibula but this operation is a more formidable procedure and the convalescence is prolonged

Treatment of Ununited Fractures of Shaft Bones with Cancellous Bone Grafts

The site of the ununited fracture is exposed subperiosteally and if necessary the fracture is freshened and reduced At this stage an intramedullary nail may be inserted in a retrograde manner and driven across the fracture line into the distal fragment to impact the fracture Strips of cancellous bone procured from the ilium as already described (v supra) are then wrapped around the site of fracture and held by suture of the muscles External splinting is always required for a short time but bony union is remarkably rapid by this method especially if the fragments are impacted In the case of the upper limb a plaster cast is applied For the femur a plaster spica is applied or alternatively the thigh is rested in a Thomas knee bed splint which allows the patient to begin quadriceps bracing almost immediately It is a mistake to allow weight bearing too soon after this operation and it should be delayed until bony union is advanced

Cortical Grafts Used as Struts and Bony Blocks

It is sometimes necessary to limit the range of movement of a joint to secure a better functioning position of the limb This is well illustrated by the Hendry (1949) bone block operation at the elbow where the graft is inserted into a slot in the humerus to limit extension by impinging on the olecranon process

The thumb may be arthrodesed in the opposed position by placing a cortical bony strut between the first and second metacarpals The use of struts in the arthrodesis of large joints is discussed later

Intra medullary Cortical Grafts

These are obtained from the tibia or ulna and are used mainly in the form of pegs which are driven across the site of fracture for the treatment of non union of certain fractures of small bones or bony processes such as the medial malleolus of the ankle the phalanges and the carpal scaphoid Similarly an epiphysis can be anchored to the metaphysis (as in slipped upper femoral epiphysis) with small cortical grafts inlaid across the epiphyseal plate

The Obliteration of Bony Cavities

The bone is exposed and the roof of the cavity removed care being taken not to weaken the shaft of the bone unduly Cancellous chips are then packed and pressed into position the skin is sutured and a padded plaster cast is applied As strength is not required os purum or anorganic bone (p 366) may be used

Arthrodesis of the Spine

Posterior Spinal Fusion with Cortical Bone Grafts The spines and laminae are exposed subperiosteally by stripping the spinal muscles from a sufficient area to be grafted The surface of the spines and laminae are roughened until fresh bleeding bone is exposed and at this stage the intervertebral facets may be excised The bone chips obtained in the process are used as additional grafting material Double cortical bone grafts are then laid in the prepared bed (Fig 122) and held in place by suture of the muscles Care is taken to have a broad area of contact between graft and bed and it may be necessary to cut the graft in a curved manner The patient is then nursed on a plaster bed or suitable apparatus such as a Strycher bed until union is firm and this usually takes about three months

Short twin tibial grafts are also used for intervertebral body fusion in cases of pro-

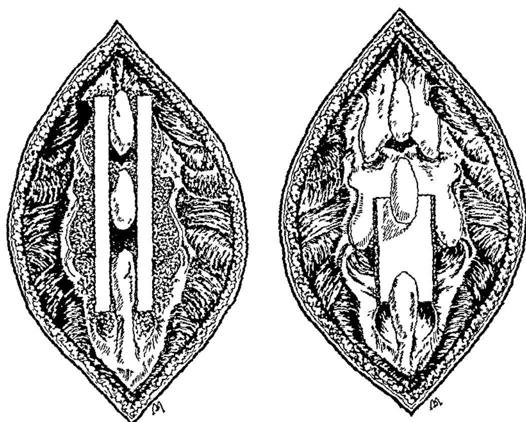


Fig 122 Posterior spinal fusion using twin cortical grafts
Fig 123 Posterior spinal fusion using an H-shaped graft from the ilium.

lapsed intervertebral disc, after removing the nucleus pulposus and the cartilage plates between the vertebral bodies Bony union tends to be slow This method has been used for spondylolisthesis, but massive posterior cortical grafts combined with grafts of cancellous bone are more popular because the operation is easier (although more arduous) and laminectomy is not required.

Fusion of the Spine with Cancellous Bone. The exposure is the same as that already described but in this case strips of cancellous bone are laid along the graft bed to overlap each other. An extensive portion of the spine may be treated, and for scoliosis it may be advisable to perform the operation in several stages.

The H-Graft. This method is useful for fusing short segments of the spine, especially in the lower lumbar region. The graft is cut from the outer aspect of the ilium and is composed of cortical and cancellous iliac bone. It is notched with bone nibblers to the form of an H and is inserted between two prepared spines and laminae in the flexed position so that on extension the graft is firmly gripped between the spines above and below (Fig. 123). The patient may be allowed up three weeks after the operation.

There is a modification of this method in which a cortical graft, called a *keyhole graft*, has a slot cut in it to receive two or more spinous processes. It may be necessary to secure the graft with stainless steel wire.

Arthrodesis of Large Joints with Cortical Grafts

Extra-articular Arthrodesis. *This method* (Fig. 124) is often used in quiescent tuberculous and pyogenic arthritis of the hip because the graft is well away from the diseased area. In the Trumble and Brittain types of arthrodesis (p. 372), *the graft is inserted into the ischium or pelvis through a femoral osteotomy.* Initial adduction of the thigh makes the operation technically easier. A plaster spica is applied until bony union is sound. A common complication of this operation is subsequent fracture of the graft, but this usually unites satisfactorily in time. The graft may also be inserted in the iliotrochanteric position as a flying buttress, if the disease is situated in the lower part of the hip and pelvis.

Intra-articular Arthrodesis. This is used mainly in the chronic rheumatic conditions and other forms of non-infective arthritis. In the case of the hip, the joint is widely exposed and dislocated by the anterior Smith-Petersen, Murphy goblet, or Kocher posterior approach and the joint surfaces are excised until raw bone is exposed. Wide discrepancies between the two may be filled *with cancellous bone and a cortical graft* is then driven or laid across the joint and may be combined with metal internal fixation. External splinting is also required.

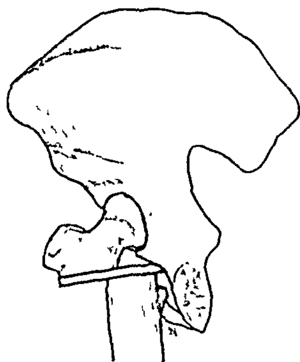


Fig. 124. Extra-articular arthrodesis of the hip of the Brittain type. The femur is divided and the shaft is displaced towards the pelvis. A massive tibial bone graft is inserted across the osteotomy and made to engage firmly with the ischium.

This type of procedure was formerly used in the knee but has now been superseded by the compression arthrodesis of Key (1932) and Charnley (1953). It is still used in arthrodesis of the hip, wrist and other joints. For arthrodesis of the ankle the distal third of the fibula may be inserted as a massive onlay graft through a lateral approach. In complete brachial plexus paralysis the function of the stump may be improved if arthrodesis of *the shoulder is combined with* amputation of the arm, and in this operation the ulna may be used as a graft and inserted through the head of the humerus into the glenoid fossa after excision of the joint surfaces. Intra-articular arthrodesis of the sacro-iliac joint is illustrated in Figure 125.

Intra-articular and Extra-articular Arthrodesis with Cancellous Bone

Many of the smaller joints may be ankylosed by the intra-articular method. A good example is arthrodesis of the subtaloid joint. The joint surfaces are excised until raw *bone is exposed and the gap is filled with* cancellous bone. Plaster fixation is maintained until bony union is sound.

Intra- and extra-articular arthrodesis are often combined. The joint is excised, and a massive tibial bone graft is placed across the joint line in an onlay or inlay position and may be held in place with metal screws. This is followed by plaster immobilization.

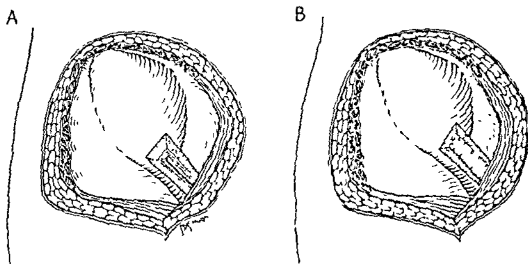


Fig. 125 Intra-articular arthrodesis of the sacro-iliac joint by Smith Petersen's method. A window is cut over the joint (A) and the articular cartilage is excised. The block of bone which was removed is replaced (after removal of its articular cartilage) and driven firmly across the joint into the sacrum (B)

The hip and wrist are often arthrodesed by this method

Treatment of Congenital Pseudarthrosis of the Tibia

Two methods have proved successful in the treatment of this intractable condition. In Boyd's (1939) method massive double onlay cortical grafts from the opposite tibia are firmly screwed on each side of the fragments bridging the fracture, and the intervening area is filled with cancellous bone In McFarland's (1951) method a cortical bone graft from the opposite tibia is placed behind the fragments without correcting the forward bowing Plaster casts are applied for many months until bony union is sound These methods have superseded the technically more difficult operation of transference of the entire fibula

Bone Grafting for Lesions of the Skull and Mandible

Defects in the Skull. Large defects may be filled by autologous split rib grafts, and these give good results, especially in children. The edges of the defect are roughened and the split ribs laid on the dura mater Defects of this membrane may be repaired at the same time with fascia lata. The grafts may be secured to the edges of the defect by drilling holes and suturing them in place with silk or wire, but this is not essential. Bony union occurs in children. In adults bony union probably does not occur, and the grafts are partly absorbed, but a sufficiently strong mass of tissue remains which gives adequate protection.

Face and Jaws. Bone grafts are used to repair defects of the malar, frontal and nasal bones, and of the mandible. These usually consist of cancellous iliac bone or ribs, and autologous bone is used for preference. It is important to make sure that there is sufficient skin cover after the contour of the skull and cheek is raised.

The bridge of the nose may be reconstructed with iliac bone grafts inserted through the columella when the bony part of the bridge line is low and the nose tip has moderate support (Gillies and Millard, 1957) The graft survives if bony union occurs, otherwise it may be expected slowly to absorb.

Extensive bone grafting procedures on

the mandible require careful planning and are not carried out until adequate skin cover has been obtained. Large gaps resulting from trauma, infection or tumours have been successfully bridged. Dental infection and other sepsis must be cleared and the buccal cavity healed. Suitably shaped grafts are cut from the crest of the ilium and wired to the roughened mandibular fragments, or the graft may be "stepped" into place as an onlay. Great care must be taken not to open into the mouth in preparing a suitable vascular bed for the graft. Post-operative splinting is carried out by wiring the teeth together or, in edentulous patients, by the multiple pin method of fixation. Splinting must not be too prolonged because the functional stimulus of mastication aids union. Split rib grafts may also be used. Short gaps in the mandible may be bridged with chips of cancellous bone.

The contour of the chin is restored later with chips of cancellous bone after the manner of Mowlem (1944).

FUTURE PROSPECTS

The extent to which bone transplants are used in the future will depend on advances in other fields of medicine and surgery, and as Neuhof and Hirshfeld (1923) have said, "the clinical value of bone grafting will be greatly enhanced by reserving the procedure for situations in which it is really indicated."

As has already been mentioned, tuberculous joints are far less often treated by arthrodesis than formerly, and poliomyelitic deformities requiring extensive grafting procedures have become less common because of the use of specific vaccines for prophylaxis.

On the other hand the number of congenital deformities will increase with the growth of the world population and the incidence of trauma is not likely to diminish. It seems therefore that these will be the main fields for the extended use of bone grafts, but their range of usefulness will depend on advances in the transplantation of tissues generally, and in particular on the development of methods of preventing or overcoming the homograft reaction, and of improved methods of sterilizing and storing bone grafts. For the moment nothing has been found to equal the fresh autologous bone graft, and when an osteogenic stimulus is required, the fresh autologous cancellous bone graft.

As we have seen encouraging results have been obtained with prepared anorganic bone, which is non-living and non-antigenic, for filling bony cavities and building up facial and alveolar contours. It remains to be seen whether this material will prove to be of value in the treatment of ununited fractures and for arthrodesis.

Attempts are being made to develop methods for hastening the union of fractures and bone grafts, but so little is known of the physiology of the osteoblast that success appears to be far distant. Bone gelatinates and granulates may offer a material for future exploitation. The notion of injecting around the site of a fracture or bone graft a sterile fluid or semi-solid osteogenic material which would set and harden immediately and provide a strong bond of union, is an attractive one and if realized would be a great advance on present methods and would have wide application, but so far the materials tried have fallen far short of this ideal. Tissue culture of pure osteoblasts may reveal the local conditions necessary for stimulating osteogenesis, but the difficulty of obtaining pure strains of adult bone cells for study has not yet been overcome.

A NOTE ON THE TRANSPLANTATION OF TEETH

Transplantation of teeth was practised by John Hunter (Fig. 126), but the results were poor and as prosthetic dentistry developed the procedure became obsolete.

Transplantation of a developed tooth involves severance of all vascular and nervous connexions, and although experimental work in animals has shown that teeth which have been removed and immediately replaced in the same socket may become firmly re-attached by regeneration of the periodental membrane (Myers, 1954), it seems unlikely that the procedure will again be used in dental practice.

Autotransplantation of a tooth germ is another matter, because prior to eruption the nutrition is provided by a network of capillaries and there is no true nerve tissue in the dental pulp (Apfel, 1954). It is being investigated clinically (Apfel, 1954; Weiner, 1956) as well as in experimental animals (Fleming, 1954).

Clinically the main indication appears to be loss, or threatened loss, of the first permanent molar in a teen-age patient, and the third molar tooth bud is transplanted. The bud is removed intact and the cavity in the bone following extraction of the first molar is enlarged to accommodate it. According to Apfel, who since 1946 has performed more than 100 operations of this kind, the transplant erupts some time within the succeeding six to nine months, and arrives in occlusion about a year after operation. As the tooth develops new roots form, and the pulp remains alive. Weiner, who also ob-



Fig. 126. Transplantation of teeth in the time of John Hunter. A contemporary picture by Thos Rowlandson (1756-1827). (Reproduced by courtesy of the President of the Royal College of Surgeons of England.)

tained good results with this procedure (Fig 127) states that eruption usually occurs within two weeks of transplantation

Homotransplantation of a tooth germ was tried by Apfel in 11 patients, but the graft sloughed in every case



Fig 127 Radiographs of a 15 year old girl whose carious permanent first molar tooth was replaced by her own impacted third molar A Before operation B Three weeks after operation C Seven months after operation D Three and one half years after operation (Dr Edward Weiner's case Figures A C reproduced from the *Transplantation Bulletin* by courtesy of the publishers and Dr Weiner Figure D by courtesy of Dr Weiner)

CHAPTER 19

Transplantation of Cartilage

PROPERTIES OF CARTILAGE

Cartilage consists of a firm resilient matrix in which there are spaces, known as lacunae, containing one, two, three, four or more cells (Fig. 128) The matrix contains chondromucin, a glycoprotein which on hydrolysis yields the sulphonated polysaccharide chondroitin sulphate (Maximow and Bloom, 1957), and is permeated by fibrils of various kinds, and the cartilage is classified as hyaline-, elastic- or fibro-cartilage according to the nature of the fibrils.

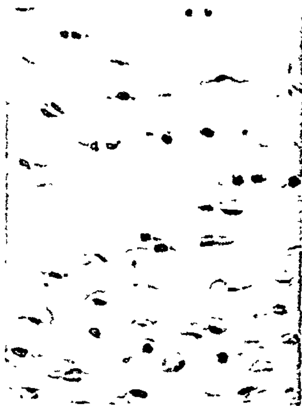


Fig 128 Normal costal cartilage from a child Haematoxylin and eosin ×400

There has been much discussion of how cartilage cells are nourished. It used to be thought that the lacunae were linked by a system of fine canaliculi, but it is now generally accepted that these are artefacts, and that nutrient fluid can reach the cells only by *diffusion* through the matrix itself (Clark, 1952; Maximow and Bloom, 1957). It seems clear that diffusion can occur over a considerable distance, but in large masses of cartilage there are sometimes canals containing blood vessels surrounded by loose areolar tissue, and there is little doubt that these are formed to meet the nutritional requirements (Clark, 1952) Such canals are common in human embryos and young children (Haines, 1933), and in the epiphyseal cartilage of man and other large mammals

Defects in cartilage are repaired by connective tissue from the perichondrium or nearby fascia, but this connective tissue may later be converted to cartilage by a process of metaplasia, apparently under the influence of local pressure and friction. As Clark (1952) has pointed out, this would seem to imply that cartilage formation is not necessarily dependent on specific chondroblasts, and Lacroix's (1949) observation that cartilage may be formed at the site of an intramuscular injection of an alcoholic extract of epiphyseal cartilage points to the same conclusion.

TRANSPLANTATION OF CARTILAGE

HISTORY*

Transplantation of Cartilage Other Than Epiphyseal and Articular Cartilage

It will be convenient to consider first transplantation of cartilage other than epiphyseal and articular cartilage. The data to be reviewed will be arranged under the following headings:

1. Animal experiments with living cartilage.
2. Animal experiments with dead cartilage.
3. Clinical observations with living cartilage.
4. Clinical observations with dead cartilage.

Animal Experiments with Living Cartilage

According to Rehn and Ruef (1921), Middledorf transplanted cartilage in experimental animals in 1852, but the transplants were all absorbed. In 1865 however Paul Bert observed that when the tail of a rat was buried subcutaneously as a free autotransplant the cartilage and bone remained alive for months, whereas the nerve and muscle soon disappeared.

Subsequent experiments by Ollier (1867), Leopold (1881), Prudden (1881), von Mangoldt (1899) and others confirmed that both autotransplants and homotransplants of adult cartilage might survive virtually unchanged for months, but many other experiments were reported in which cartilage transplants were quickly absorbed. Ollier (1867) and Fischer (1882) believed that the presence of perichondrium was necessary to ensure survival.† Prudden (1881) found however in rabbits that homotransplants from which the perichondrium had been completely removed were able to survive for many months, and Davis (1913, 1917a) found that this was true also of autotransplants of cartilage in dogs.

Zahn (1877) initiated experiments with homotransplants of foetal cartilage, and observed that such transplants not only survived but grew. Subsequently Leopold (1881) made an extensive study of transplants of foetal cartilage to the abdomen, the jugular vein and the anterior chamber of the eye. He found that both homotransplants and heterotransplants increased in size, the former to as much as 300 times their original volume over a period of 200 days.

Mannheim and Zypkin (1926) found that autotransplants of adult cartilage in rabbits survived longer in soft tissues than when placed in defects in bone, and longer in muscle than in subcutaneous tissue. They reported also that survival was actually facilitated by partial removal of the perichondrium.

Loeb and his colleagues (Loeb, 1926a, b, c, 1927b, 1945; Loeb and Harter, 1926; Loeb and Siebert, 1935) made a comparative study of the behaviour of autotransplants, homotransplants and heterotransplants of cartilage in guinea pigs, rats, and chickens. Autotransplants were observed to survive virtually unchanged for many months. Homotransplants evoked a lymphocytic and fibroblastic reaction, but despite this they, too, often survived for long periods, and in one experiment in rats a piece of cartilage was maintained by serial homotransplantation for more than six years. Homotransplants evoked less reaction when the donor and host were closely related. Heterotransplants (Loeb and Harter, 1926; Loeb, 1945) were found to evoke a much more violent reaction than homotransplants, and were absorbed within a few weeks.

Kirkham (1940) found that rabbit auricular cartilage stored in Ringer's solution on ice for 24 or 48 hours, and then transplanted

*In preparing this section much help has been obtained from reviews by Dupertuis (1941), Peer (1939b), and Rehn and Ruef (1921).

†In Ollier's words "le tissu cartilagineux dépouillé de son périchondre, ne se greffe pas."

subcutaneously to another rabbit, still retained the physical characteristics of cartilage when removed six months later, whereas cartilage kept dry at the same temperature and for the same period failed to do so. The cells did not survive, however, even in transplants stored in Ringer's solution

Dupertuis (1941) made an extensive study of autotransplants, homotransplants and heterotransplants of cartilage in rabbits, with particular reference to the conditions under which growth occurred He found that subcutaneous autotransplants of ear cartilage in young rabbits (2-3 weeks old) increased in length on average by 122 per cent in the course of 161 days, while with intramuscular autotransplants in animals of the same age the growth rate was even greater In both cases the transplants maintained the histological appearance of healthy cartilage, and showed no evidence of lymphocytic infiltration. Homotransplants of ear cartilage from young donors to young hosts increased in length on average by 56 per cent in the course of 173 days, but after being *in situ* for this length of time stained somewhat irregularly, and showed focal necrosis and lymphocytic infiltration. The findings were much the same when litter mates were used as when the donor and host were of different breeds Homotransplants of ear cartilage from adult rabbits to young hosts did not grow and evoked a moderate fibroblastic reaction in the surrounding host tissue On the other hand homotransplants of ear cartilage from young donors to adult hosts increased in length by as much as 95 per cent in the course of 55 days, though they evoked some degree of lymphocytic reaction. Autotransplants and homotransplants of rib cartilage behaved very much as corresponding transplants of ear cartilage, except that the rate of growth was never as great.

Young (1941, 1945) studied subcutaneous transplants of costal cartilage in dogs. Fresh autotransplants maintained their size, weight and microscopic structure during the 18 months they were left *in situ*, irrespective of whether the perichondrium was retained or not. Central calcification was often observed, and sometimes ossification, while occasionally bone marrow was formed as well as bone. Homotransplants did not change in size or macroscopic appearance, but on microscopic examination some of the cells appeared to have lost their nuclei.

Bacsich and Wyburn (1947), in experiments in guinea pigs, made simultaneous homotransplants of cartilage and oesophagus, liver, spleen or ovary, from the same donor to the same recipient. The transplants were explored after three weeks, by which time the cartilage showed no gross change, and on microscopic examination appeared healthy except for small scattered areas of necrosis, whereas the other tissues had either disappeared, or showed profound alterations in structure and evidence of lymphocytic or fibroblastic reaction on the part of the host. Bacsich and Wyburn suggested that the relatively long survival of homotransplants of cartilage was due to the protective action of the mucopolysaccharides of the ground substance (p. 52).

Animal Experiments with Dead Cartilage

Prudden (1881) studied the behaviour of homotransplants of dead cartilage in rabbits The transplants, which were killed by drying, exposure to alcohol or carbolic acid, boiling, or passage of a strong electric current, became surrounded by, and subsequently permeated by, granulation tissue, and were usually completely absorbed in the course of a few weeks.

Seggel (1904) confirmed that cartilage which had been preserved in alcohol was absorbed more rapidly than fresh cartilage, and Nageotte (1917a, b, d) reported that while alcohol-preserved cartilage might persist unchanged for a long time it was liable to be suddenly destroyed by the digestive action of ferments liberated by polymorpho-

nuclear leucocytes Polettini (1922) Nigrisoli (1927) and Didier and Guyon (1928) observed that new cartilage and bone was sometimes formed in the vicinity of transplants undergoing absorption

Dupertuis (1941) studied the behaviour in rabbits of autotransplants homotransplants and heterotransplants of cartilage which had been stored in alcohol or merthiolate or freeze dried He found that autotransplants and homotransplants removed after several months showed various degrees of absorption rib cartilage as a rule surviving better than ear cartilage Preserved heterotransplants of dog cartilage evoked a more intense lymphocytic reaction than either autotransplants or homotransplants

Segments of rat and guinea pig trachea were used experimentally as homotransplants by Rob and Eastcott (1951a) All the frozen transplants became necrotic but as Rob and Eastcott point out this failure cannot fairly be attributed to the method of storage because only one fresh homograft out of eight worked entirely satisfactorily

Clinical Observations with Living Cartilage

It is uncertain who was the first to transplant cartilage in man but among the earliest to do so was Konig (1896) who used a compound flap of skin and laryngeal cartilage to repair a defect in the trachea remaining after tracheotomy performed some years previously for laryngeal diphtheria

Von Mangoldt (1899) successfully treated stenosis of the larynx by transplanting autologous costal cartilage subcutaneously to the submental region and subsequently fashioning a compound flap of skin incorporating this cartilage and inserting it between the thyroid cartilages after performing laryngofissure

Nelaton and Ombredanne (1904) Henle (1904) Bunnie (1908) Davis (1913 1917b) Carter (1917) and others used autologous costal cartilage in reconstructive operations on the nose

Weglowski (1907) after excising a synostosis of the elbow placed two pieces of autologous costal cartilage between the bone ends Each graft was covered on one side with perichondrium and this was applied to the humerus The patient died of pneumonia five weeks later but before he died he was able to move the joint through 60 70° and at autopsy the grafts appeared healthy and were firmly fixed to the humerus

Gillies (1920a) in his book on plastic surgery of the face wrote that cartilage for large cosmetic purposes stands unrivalled It is available in sufficient quantity is easily fashioned to the desired shape and what is more important remains permanently in the shape and size in which it is imbedded with the exception that if one perichondral surface only is left the graft tends to bend the perichondrium occupying the concavity and this property of cartilage is utilized by the surgeon to obtain a curve in such positions as the eyelids or the mandible He pointed out that infection might result in necrosis of the transplant but otherwise autotransplants which he observed for up to three years survived unchanged (except for the tendency to curve towards the perichondral surface referred to above) Homotransplants though they were sometimes slowly replaced by fibrous tissue also survived well as a rule

Neuhof and Hirshfeld (1923) from an extensive survey of the literature concluded that cartilage grafts maintained their histological structure for many weeks but might subsequently calcify, be replaced by fibrous tissue or disappear They considered that the fate of a cartilage graft depended primarily on the site in which it was placed and did not draw any distinction between autografts and homografts

Peer (1939b) buried pieces of autologous costal cartilage devoid of perichondrium subcutaneously in the chest wall

undergoing rhinoplasty or repair of depressions in the skull, and removed them after periods ranging from six months to six years. He found that the cartilage became surrounded by a dense capsule of connective tissue, but maintained its normal structure and showed no evidence of absorption or invasion. He showed later that subcutaneous autotransplants of nasal septal cartilage (Peer, 1941, 1945), and also "diced" grafts (i e grafts in the form of numerous small pieces packed closely together) of autologous costal cartilage (Peer, 1943) behaved similarly.

Further experiments by Peer and Walker (1951) confirmed that cartilage autotransplants in man may survive and retain their structure irrespective of whether they are placed in contact with unlike tissue, as in the previous experiments, or in contact with cartilage

There has been a good deal of discussion as to whether growth occurs in cartilage autotransplants in young subjects. Peer (1946) claimed that it did, and confirmatory evidence was reported subsequently by Dupertuis (1950), later however Peer (1955) stated that he no longer believed this to be true

In recent years autografts of fresh cartilage have been widely used for correcting saddle deformities of the nose Herbert (1940) used ear cartilage for this purpose, Goldman (1940) used nasal septal cartilage, Carter (1932) used costal cartilage with attached bone, while Dupertuis (1950) and many other surgeons have used costal cartilage alone It has been found however that, contrary to the belief of Gillies (p. 385) and others, the grafts sometimes become distorted (*see* Fig 129)

Peer (1944), Ivy (1944) and Young (1944) pioneered the use of cartilage for restoring the facial contour in patients with a variety of bony deformities and for reconstruction of the pinna (*see* Fig. 131). Young used grafts of two kinds The first, which he called a cast graft, consisted of diced cartilage introduced into the defect, roughly moulded by external pressure, and covered by a previously prepared vulcanite splint shaped to conform accurately to the corrected contour required. The second, which Young called a pre-cast graft, consisted of diced cartilage placed in a fenestrated metallic mould and buried subcutaneously in the patient before being transferred to the defect Pre-cast grafts were subsequently used extensively by Peer (1948).

Dufourmentel and Darcissac (1935) interposed autologous cartilage between the bone ends after division of the mandible in order to produce a false joint in patients with ankylosis of the temporo-mandibular joint. This operation was employed subsequently by Padgett, Robinson and Stephenson (1948), and Longacre and Gilby (1951). Pickerill (1942) achieved much the same result by inserting two pieces of autologous cartilage after excising the condyle of the mandible.

Brodkin (1954) used diced autologous cartilage to fill the residual depression after performing chondro-sternoplasty for the correction of congenital funnel chest.

Fresh homologous cartilage, as distinct from non-viable stored homologous cartilage, has not been widely used except in the treatment of congenital absence or deformity of the external ear. Gillies (1937), Greeley (1941) and Mowlem (1941) used maternal cartilage for this purpose, and obtained some excellent results. Kazanjian and Converse (1949) used paternal cartilage in one case, but 15 months later it was found that the graft had become softened and was in process of being destroyed.

Clinical Observations with Dead Cartilage

Autografts. Cartilage autografts are usually viable, but New and Erich (1941) used autografts which had been heated to 96°C. in either water or a solution of merthiolate for correcting nasal deformities. They

claimed that such grafts were less liable to undergo distortion than viable autografts

Homografts. The first account of the clinical use of homografts of preserved non living cartilage appears to be that of O Connor and Pierce (1938), but many years before their paper was published, as Peer (1955) has pointed out, otorhinolaryngologists began to use homografts of septal cartilage preserved in alcohol for correcting saddle deformity of the nose following submucous resection of the septum

O Connor and Pierce stored their grafts in a refrigerator in a mixture of one part of a 1.1000 aqueous solution of merthiolate and four parts of physiological saline They reported that there were only 7 unsatisfactory results in 375 patients observed for up to 5 years, and that the preserved grafts showed less tendency to curl, and appeared to be more resistant to infection, than fresh autografts (O Connor and Pierce, 1938 O Connor, 1939, 1940) Straith and Slaughter (1941) also reported favourably on merthiolate preserved cartilage, which they used in the treatment of a variety of contour defects of the face including the nose

Peer (1938), on the other hand, found that homologous cartilage preserved in alcohol and subsequently transplanted experimentally to the chest wall evoked a foreign body reaction and was gradually absorbed, and Brown (1940), who used frozen and alcohol preserved homologous cartilage in reconstructive operations similar to those of Straith and Slaughter, concluded that this material was less resistant to infection than fresh autologous cartilage, and was also liable to be absorbed in the absence of infection

Despite these admittedly serious disadvantages stored non living cartilage has continued to be widely used in virtually all the operations in which fresh autologous cartilage is used, including the restoration of contour in the nose and elsewhere (Vidaurre 1952, Schofield, 1953), and the repair of large defects in the chest wall (Brodkin and Peer, 1954), mainly because it can be obtained readily in large quantity Cadaver cartilage preserved in merthiolate is the material most commonly used (see e g Brown and De Mere, 1948), but some surgeons have used semilunar cartilage from the knee obtained at operation (Mir y Mir, 1952, Vidaurre, 1952)

Today there is a tendency to prefer to use fresh autologous cartilage but, as Schofield (1953) has pointed out, non living cartilage is perfectly satisfactory for repairing contour defects in children, in whom it may be necessary to insert successive grafts as they grow

Heterografts. Stout (1933) appears to have been the first to use heterografts of non living cartilage in man. He preserved ox cartilage in formalin and, after soaking it in sterile water to get rid of the formalin, used it in nasal reconstruction The short-term results were satisfactory, but at the time this work aroused little interest More recently, however, ox cartilage immersed for a brief period (1 15 minutes) in boiling water and stored in a 1 1000 solution of merthiolate has been used by Wardill and Swinney (1947), Gillies and Kristensen (1951), Braithwaite and Hopper (1952), and Gibson and Davis (1953)

Gillies and Kristensen, in reviewing 111 operations performed on 125 patients, concluded that the probability of success was 95 per cent in grafting to the nose, 50 per cent in grafting to the ear, and 80 per cent in restoring other facial contours, and claimed that these figures did not differ significantly from "what one would expect from any other grafting material in these regions"

Gibson and Davis, whose technique differed from that of Gillies and Kristensen only in that they used cartilage from the scapula instead of from the xiphisternum

and boiled it for 10-15 minutes instead of for 1 minute, were less optimistic. They found that the short-term results were excellent, but after about 18 months some degree of absorption was the rule, and within 2 years many grafts had disappeared entirely. Absorption began as surface erosion and this was followed by fibrous replacement and liquefaction in varying degree. In one patient—a boy with congenital absence of the external ears—diced bovine cartilage in a vitallium mould was buried in the abdominal wall and subsequently transferred to the pre-auricular region. The graft was slowly absorbed and a second graft of the same material was therefore inserted. This was absorbed much more rapidly than the first graft, and experiments performed subsequently in volunteers suggested that second grafts were destroyed more rapidly than first grafts as a general rule if they were obtained from the same breed of cattle, though not necessarily when the two grafts were obtained from cattle of different breeds.

Braithwaite and Hopper (1952) inserted merthiolate preserved bovine cartilage between the bone ends after dividing the ascending ramus of the mandible in five patients with ankylosis of the temporo-mandibular joint, with the object of producing a pseudarthrosis. The results were very satisfactory, but this does not necessarily imply that the grafts persisted.

Transplantation of Epiphyseal Cartilage

Animal Experiments

It was shown by Ollier (1867) that autotransplants of epiphyseal cartilage were able to ossify, and this has since been confirmed by many observers. More recently Pereira and Dupertuis (1936) have shown that such transplants may give rise to typical exostoses.

It has proved much more difficult to determine with certainty whether orthotopic transplants of epiphyseal cartilage retain their property of producing longitudinal growth in the bone in which they are situated, and the published observations are conflicting.

Enderlen (1899) excised and then replaced the distal epiphyseal cartilage of the ulna, with an adjoining piece of epiphysis and diaphysis, in rabbits. He observed that the central part of the epiphyseal cartilage degenerated but the peripheral part retained its microscopic structure, and von Helferich (1899), reporting on the same experiments, stated that under favourable conditions the epiphyseal cartilage retained its property of producing longitudinal growth, though this property might be impaired. Confirmatory reports were published by various German investigators, including von Tappeiner (1913), who transplanted the distal half of the metatarsal in dogs, and Heller (1914, 1917) and Fohl (1929), who transplanted the distal radial or ulnar epiphysis in rabbits.

In the United States, on the other hand, Haas (1915, 1916, 1931b) observed little or no longitudinal growth following autotransplantation of epiphyseal cartilage, with or without a piece of epiphysis or diaphysis, in either dogs or rabbits, and Bisgard (1939) also obtained negative results in goats. In Bisgard's experiments a slice consisting of distal epiphyseal cartilage from the femur and a thin piece of bone on each side was interposed as an autotransplant in the tibia after excising a disc of bone of equal thickness, and bridges of new bone soon grew across the gap making any subsequent increase in length impossible. Haas, however, followed very closely the various experimental procedures described by the German investigators, and the discrepancy between his findings and theirs is difficult to understand.

Homotransplants of epiphyseal cartilage were studied in dogs by von Tappeiner (1913), and in rabbits by Rehn and Wakabayashi (1912), Heller (1914, 1917) and von

Lippmann (1916). Rehn and Wakabayashi transplanted the head of the radius including the upper radial epiphysis between litter mates at the age of two months and reported that the transplanted epiphyseal cartilage survived and functioned; this conclusion was based entirely on radiological evidence, however, and has been strongly criticised by Haas (1915). Von Lippmann in similar experiments reported that the transplants *functioned in two instances* but in none of the other experiments cited was any longitudinal growth observed which could be attributed to activity *of the transplant*.

Clinical Observations

Rehn and Wakabayashi (1912), encouraged *by the results of their experiments in* rabbits, after resecting the ulna in a girl aged 4 years replaced it with two portions of fibula from a freshly amputated limb. *The central part of the shaft of the fibula* was discarded but both ends were retained, the distal end being located at the elbow and the proximal end at the wrist. Three *and a half months after the operation move*ment at the wrist was almost normal and the patient was able to flex the elbow to 90° and to fully extend it. There is however no *record of whether or not the transplants sub*sequently increased in length.

More recently Straub (1929) reported a case in which he used an autotransplant. *The patient was a boy aged 6½ in whom* two years previously the distal half of the left tibia including the epiphysis had sequestrated as a result of osteomyelitis. There was *in consequence gross inversion of the foot* due to the continued growth of the fibula and to correct this the missing bone was replaced by a graft from the opposite tibia. This graft was 13 *cm. long and included* the anterior part of the medial malleolus and the medial portion of the epiphyseal plate. The result was described as excellent. In particular *some 11 years later the* patient was free from pain, the calcaneo-cavus deformity, though it had not been *completely corrected*, was very *much less* than before, and there was only slight limitation of flexion and extension at the ankle joint. Straub considered that longitudinal *growth had occurred in the transplant*, but Haas (1931b) subsequently pointed out that there was no definite evidence of this, and that the good result could be accounted for by compensatory growth at the *upper tibial* epiphysis.

Wenger (1945) replaced the left first metatarsal bone of a 7 year old boy, which had *been completely destroyed as a result of* chronic osteomyelitis, with an autologous graft consisting of one epiphysis and part of the shaft of the fibula. The boy was kept under observation for 3 years after the operation and the functional result was excellent. The proximal part of the transplanted epiphysis fused with the adjacent cuneiform bone but this did not cause any impairment of growth and the transplant not only increased in width but grew longitudinally at the same rate as the normal right first metatarsal.

Transplantation of Articular Cartilage and Joints

Definitions

In this section we shall consider the transplantation of articular cartilage alone or together with related structures including bone, ligament and synovial membrane. Following Lexer (1908d) the terms *half joint transplantation* and *whole joint transplantation* will be used to denote respectively orthotopic transplantation of one of two bones taking part in the formation of a joint, and transplantation of the articular ends of both bones together with some or all of the ligaments uniting them.

Animal Experiments

Judet (1908) performed numerous experi*ments in rabbits. He found inter alia* that subcutaneous homotransplants of articular

cartilage with accompanying synovial membrane, and of the entire knee joint complete with capsule, retained their structure, whereas subcutaneous homotransplants of articular cartilage alone, and of the knee joint without its capsule, failed to do so. Further experiments with heterotopic transplants were reported subsequently by Axhausen (1909b, 1912), who refuted Judet's assertion that homotransplants of articular cartilage would not survive in the absence of synovial membrane, Dalla Vedova (1911), Impallomeni (1911), Ducuing (1912) and Voronoff (1916)

Judet (1908) also studied orthotopic transplants of articular cartilage and related structures. He found that free autotransplants of articular cartilage placed within the cavity of a joint survived. Likewise, autologous half-joint and whole-joint transplants placed within the capsule of the knee joint survived and functioned, but homotransplants failed to do so.

Obata (1914), also using rabbits, studied orthotopic autotransplants and homotransplants of a metatarsophalangeal joint, and orthotopic homotransplants of the head of the radius with its articular cartilage. In all cases some shortening occurred and the cartilage showed degenerative changes followed by various degrees of regeneration The whole joint autotransplants gave the best results Half joint homotransplants between siblings were fairly satisfactory, but whole joint homotransplants yielded poor results irrespective of whether the donor and host were siblings or not.

Gill (1915) interchanged the right and left second metacarpals in dogs, thereby performing four half-joint autotransplants simultaneously Seven to nine months later in approximately half the animals the joints were functioning satisfactorily and the grafts were found to have retained their normal structure.

In recent years the behaviour of whole-joint autotransplants and homotransplants has been re-investigated by Herndon and Chase (1952). These workers excised the distal third of the femur and the proximal third of the tibia, together with the collateral and cruciate ligaments and the menisci, in dogs and then inserted either an autotransplant or a homotransplant of similar structure. The bones were divided step-cut fashion, and were re-united with either screws or wire sutures. In the autotransplants the bone was quickly revascularized and dead bone was replaced by new. The articular surface of the cartilage showed degenerative changes and cellular necrosis, but in the deeper layer the cells remained viable The functional results, judged by observations extending over two years, were excellent. The homotransplants on the other hand, though they usually united and functioned for a short time, soon showed gross degenerative changes, and eventually became completely disorganized.

Clinical Observations with Transplants of Articular Cartilage Alone

Delbert (1912) and Mauclaire (1913) used free autotransplants of articular cartilage in performing arthroplasty of the elbow joint and reported that the results were encouraging.

Ostrowski (1922) studied the behaviour of heterotopic (subcutaneous or intramuscular) homotransplants of articular cartilage obtained from arthrodesis operations on crippled limbs. He found that degenerative changes occurred during the first eight weeks after transplantation but were followed later by a considerable degree of regeneration.

Pridie (1958) successfully treated osteochondritis dissecans of the femoral condyle by removing the loose body, packing minced autologous bone into the cavity, and covering the bone with a free graft of articular cartilage from the opposite condyle. Subsequently however he found that equally satisfactory results could be obtained simply

by removing the loose body and then drilling holes in the bone forming the floor of the cavity to stimulate healing

Clinical Observations with Half-joint Transplants

Autotransplants Tuffier (1901, 1911) in treating a fracture of the surgical neck of the humerus removed the head of the humerus temporarily and replaced it in a more favourable position Similar operations according to Lexer (1924 1925b) were performed by Konig and in more recent times a very successful case in which this procedure was used has been reported by May (1912)

Tietze (1902) replaced the distal end of the radius by the proximal phalanx of the great toe the base of the phalanx being located at the wrist At the time however interest centred on the fate of the transplanted bone and the subsequent degree of function in the wrist joint is not known

Wolff (1911) appears to have been the first to use a bone from the foot to replace one of the phalanges in the hand His operation which yielded an excellent functional result was performed in 1909 and numerous other cases were reported during the next few years by Leonte (1912) Goebel (1913) Sievers (1913) von Stubenrauch (cited by Lexer 1924) and others (*see* Gill 1915)

According to Lexer (1925b) Rehn used the head of the radius to replace the lower end of the same bone and Bardenheuer used the head of the fourth metatarsal to replace the condyle of the mandible

Rovsing (1910) used the upper end of the fibula to replace the head and proximal two thirds of the shaft of the humerus and this procedure has subsequently been used successfully by Borchus (1914) Skillern (1920) Albee (1921 1936) Lexer (1924 1925b) and Behrend (1930)

Homotransplants Lexer (1908c d) resected the upper part of the left tibia including the articular surface on account of a sarcoma and replaced it by a transplant from a freshly amputated right leg Apart from the fact that it was taken from the opposite side of the body the transplant was similar in form and size to the bone it replaced The ligamentum patellae and joint capsule of the host were sutured to the tibial tuberosity and periosteum of the transplant respectively but no attempt was made to reconstitute the cruciate ligaments When examined *five months after the operation the joint was* stable flexion and extension were almost normal and the radiological appearances were satisfactory A year later the function of the knee was still good but amputation had to be performed because of religious considerations of the patient (Lexer 1925b)

Soon afterwards Lexer (1908c d) resected the upper two thirds of the left humerus in a young man for sarcoma and replaced it with the lower part of the femur from an amputated limb One of the femoral condyles after being carved to resemble the head of the humerus was placed in the glenoid *fossa and kept in place by passing a purse* string suture through the margin of the *joint capsule and then fastening the capsule* to the bone by means of a few nails The transplant was fixed to the humerus of the host with a bone peg fashioned from the fibula of the amputated limb

In a third patient Lexer replaced the proximal phalanx of a finger with a phalanx from the foot of an amputated lower limb

Subsequently Lexer himself and various other European surgeons including von Haberer (1911) Duval (1913) Oehlecker (1916 1922) and Kuttner (1910 1911) performed numerous homologous half-joint transplantations and in reviewing these cases some years later Lexer (1924 1925b) concluded that the operation offered an excellent chance of restoring satisfactory function provided that after transplantation to the lower limb weight bearing was post

poned for sufficiently long to permit revascularization and regeneration of the subchondral spongy bone.* Kuttner obtained most of his transplants from cadavers, but the other surgeons referred to as a general rule preferred to use transplants from amputated limbs.

Another success was claimed by a Scottish surgeon, Wade (1920), who reported full shoulder movement in a patient in whom he had replaced the head, neck and upper part of the shaft of the humerus with the lower end of the femur from an amputated limb seven years previously.

More recently cases have been reported by May (1942) and Capanno and Pedemonte (1953).

May replaced the upper third of the tibia of a young woman with a corresponding portion of tibia from an elderly man of the same blood group (O). The patient recovered some movement in the knee but on exploration two years later it was found that the bulk of the graft was dead.

Capanno and Pedemonte excised the entire femur from a man aged 41 on account of gross hydatid disease and replaced it with the femur of a young girl killed in an accident. The graft was removed from the donor within two hours of death. Two months after the operation the patient had a few degrees of movement in the knee and slightly more in the hip. Ten months after operation he was walking confidently but the knee was almost completely stiff. A biopsy at this time showed that much of the graft was dead, but there was new living bone beneath the periosteum and bone marrow in the medullary cavity.

Clinical Observations with Whole-joint Transplants

Autotransplants. Buchmann (1908) reported two cases of bony ankylosis of the elbow in which mobility was restored by transplanting the entire first metatarsophalangeal joint complete with its capsule, and Goebell (1913) transplanted an interphalangeal joint from a toe to a finger.

Much more recently Peer (1955), after removing a complete supernumerary toe and a complete supernumerary finger, buried these structures subcutaneously in the patient's abdominal wall as an experiment. Two months later the joints could be passively flexed and extended, and radiological examination revealed normal bone structure.

Homotransplants. Between 1907 and 1925 Lexer performed whole-joint homotransplantation in 23 patients (Lexer, 1906, 1909, 1921, 1925b). Eighteen of these patients received knee joints, transplanted, except in one instance, without the capsule.

Lexer reported that 12 patients were permanently cured, but, while some of the results were surprisingly good, this claim is exaggerated. Nine of the 12 patients had received knee joints, and six of the nine were followed "for at least a few years," during which time they had little or no pain and recovered a considerable degree of mobility. Radiological examination, however, revealed progressive osteoarthritic change, and in two cases in which the transplants were examined post mortem by Burkle, after being in place for 11 and 16 years respectively, it was found that the articular cartilage had been entirely replaced by fibrous tissue. The other three patients reported as cured had received finger joints. They were discharged from hospital "after good healing with painless but not yet completely normal mobility" (Lexer, 1925b), but unfortunately were not examined subsequently.

CURRENT VIEWS ON THE BIOLOGY OF CARTILAGE GRAFTS

The observation of Billingham and Boswell (1953), that homografts of skin placed

*In this connexion Lexer wrote in 1925: "Today I would use a weight diverting apparatus on a leg for at least two years."

in pockets in the host's cornea survive indefinitely so long as they do not become vascularized suggests that the *long survival of homografts of living cartilage* may be accounted for by the fact that they are avascular and are not readily penetrated by host cells.

This hypothesis gains strong support from the work of Algire and his colleagues (Algire, Weaver and Prehn, 1954, 1957; Prehn, Weaver and Algire, 1954; Weaver, Algire and Prehn, 1955), Woodruff (1957b) and others on the behaviour of homografts of various tissues isolated from contact with host cells by permeable membranes such as 'Millipore', and from Woodruff's (1957f) demonstration that the necessary and sufficient condition for the mammalian foetus to escape the immunological hazard of pregnancy is that it should be effectively isolated by the placental barrier from contact with maternal cells (p. 193).

The ground substance may well be important in this connexion in that it may help to determine the avascularity of cartilage and its resistance to cellular infiltration. There does not seem to be any justification, however, for postulating, as Bacsich and Wyburn (1947) have done, that the mucopolysaccharides of the ground substance exert some more subtle protective effect.

Homografts of *dead* cartilage almost certainly contain no H antigens (p. 93) and could therefore not be expected to evoke a homograft reaction. This does not explain why for so long they escape destruction by phagocytic cells, but once again the special properties of cartilage discussed above would seem to provide a satisfactory explanation.

Observations on the behaviour of heterotransplants of various tissues isolated from contact with host cells suggest that the factors which determine the behaviour of cartilage homografts are responsible also for the long survival of heterografts of living cartilage and the persistence of heterografts of dead cartilage. Both types of heterograft contain heterologous antigens (p. 115) but these antigens apparently do not escape to any significant extent so long as infiltration by host cells is prevented.

TRANSPLANTATION OF CARTILAGE IN CLINICAL SURGERY: PRESENT STATUS AND FUTURE PROSPECTS

In this section we shall discuss the transplantation of cartilage in clinical surgery under the following headings:

1. Restoration of normal contour.
2. Repair of large defects in the chest wall and elsewhere.
3. Reconstruction of the pinna.
4. Arthroplasty and transplantation of joints.
5. Transplantation of epiphyseal cartilage.

Restoration of Normal Contour

Cartilage grafts are very useful for restoring normal contour in patients with depressions in the bones of the skull, including the frontal and nasal bones, the floor of the orbit, and the mandible. They are also used to correct saddle deformities of the nose, though many surgeons, including Mowlem (1941), prefer to use bone grafts for this purpose owing to the distortion which sometimes occurs in cartilage grafts (Fig. 15).

In adults autologous cartilage gives the best results and is available in considerable quantity in the form of costal cartilage. The removal of costal cartilage is quite a major procedure, however, and great care must be taken to avoid infection at the donor site.

Homologous cartilage stored at 4° C. in saline containing 1 part in 4,000 of merthiolate is a useful substitute when autologous cartilage is not available in sufficient quantity, and is probably the material of choice in children when it seems likely that suc-

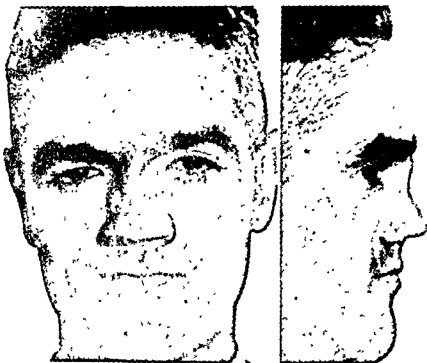


Fig 129 Photograph showing gross distortion of an originally straight cartilage graft (Reproduced from the *British Journal of Surgery* by courtesy of the publishers and Mr Rainsford Mowlem)

cessive grafts will be required as the patient grows

Heterologous cartilage is much less satisfactory and should not be used if homologous or autologous grafts can be obtained If heterologous cartilage is used it should be boiled for a few minutes and subsequently stored in merthiolate solution in the same manner as homologous cartilage (*v. supra*).

Repair of Large Defects in the Chest Wall and Elsewhere

Diced merthiolate-preserved homologous cartilage is useful for repairing large defects in the chest wall. The technique of Brodkin and Peer (1954), who were the first to use cartilage for this purpose, is illustrated in Figure 130

Peer has advocated the use of diced cartilage to provide reinforcement in repairing extensive or recurrent herniae, and in the treatment of spina bifida when there is a large bony defect in the spinal canal, but it seems unlikely that these operations will ever be widely used. In the treatment of hernia alternative methods of repair, for example the insertion of nylon or tantalum mesh, are simpler and very satisfactory. In spina bifida occulta no repair of the bony defect is required, and in more serious forms of spina bifida there is often such extensive paralysis of limb and sphincter muscle, associated with sensory loss, that surgical treatment is not justified.

Reconstruction of the Pinna

Congenital absence or deformity of the pinna presents a difficult problem in treatment One solution, which has given good results in experienced hands, is to bury diced autologous cartilage in a perforated vitallium mould beneath the skin of the patient's abdomen until such time as the grafts have become welded into a solid mass by ingrowing connective tissue,* and then transfer the

*The mould is usually left in place for about five months

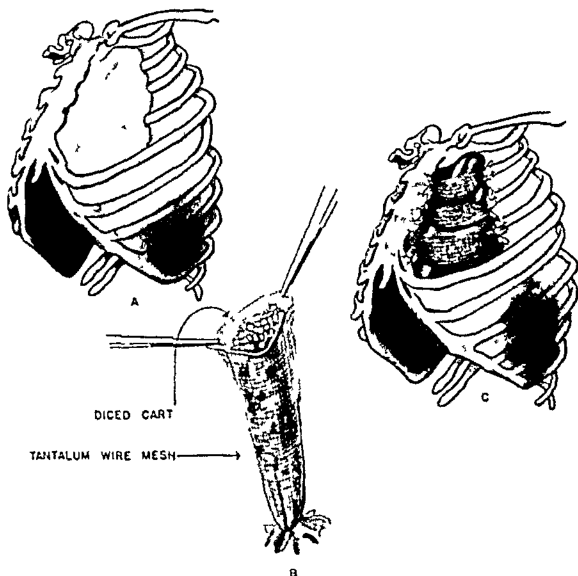


Fig. 130. Technique of Brodkin and Peer for repairing a large defect of the chest wall (A). Bags made of tantalum mesh are filled with diced homologous cartilage (B) and used to replace the missing pieces of rib (C). (Reproduced from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Dr Lyndon Peer.)

contents of the mould to a previously developed subcutaneous pocket in the auricular region (Fig 131). Alternatively a single piece of maternal cartilage carved to the proper shape may be used.

Arthroplasty and Transplantation of Joints

In ankylosis of the temporo-mandibular joint a false joint may be created in the ascending ramus of the mandible by resecting a portion of bone and replacing it by a free graft of cartilage. Good results have been obtained with merthiolate preserved bovine cartilage (Braithwaite and Hopper 1952) but it would seem safer *to use homologous* if not autologous material. An alternative method of treatment is to resect the condyle of the mandible and replace it by a cartilage graft.

Attempts have been made (Peer 1955) by burying diced cartilage grafts in a suitable mould to make a cup of cartilage which could be used to cover the head of the femur

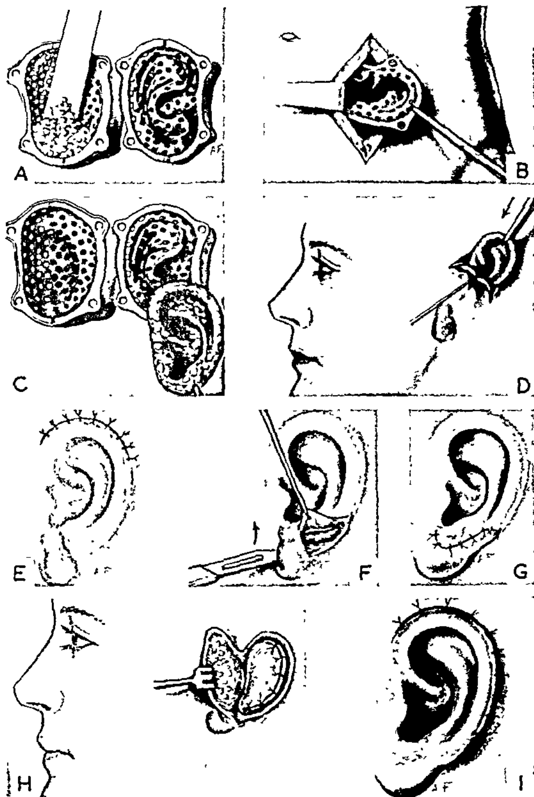


Fig 131 Peer's method of reconstructing the auricle with diced cartilage grafts. An ear mould made of perforated vitallium is filled with diced homologous cartilage (A) and buried subcutaneously in the abdominal wall (B) Five months later the mould is removed and the cartilage, which has become fused into one solid mass (C), is inserted into a previously delayed pocket in the auricular region (D, E) ' the ear lobe is brought into normal relationship with the reconstructed auricle (F, G). Still composite cartilage graft is dissected free from the temporal fascia and the periosteum of the bone, and a split-skin graft draped over a mould of dental stent is inserted into the sulcus thus ... (H, I). Finally, one or more operations are performed to sharpen the helix, increase the depth of th concha, and replace hair-bearing by hairless skin (Reproduced from *Plastic and Reconstructive Surgery* by courtesy of the publishers and Dr. Lyndon Peer.)

when performing arthroplasty of the hip. This procedure does not appear to have been used outside Peer's own clinic, but merits further investigation. It would seem worth while also to determine whether grafts of articular cartilage backed by a thin shaving of bone could be used successfully for the same purpose.

Transplantation of large joints is now entirely obsolete, and appears unlikely ever to be revived.* On the other hand, transplantation of small joints, and especially autotransplantation of an interphalangeal joint from a toe to a finger, though not very often performed today, may well become firmly re-established in surgical practice.

* The use of metal and plastic prostheses in arthroplasty is another matter, but does not fall within the scope of this book.

Transplantation of Epiphyseal Cartilage

Attempts to re-establish growth in a long bone by autotransplantation of an epiphysis have yielded such disappointing results that this operation is rarely performed today. As we have seen, however, a few successful cases have been reported, and these should stimulate further experimental work.

CHAPTER 20

Transplantation of Cornea

By George I. Scott

STRUCTURE AND FUNCTION OF THE CORNEA IN RELATION TO TRANSPLANTATION

Structure

The cornea and the sclera constitute the fibrous envelope of the eye, the cornea possessing in its transparency a unique quality; the factors which are responsible for this quality have yet to be elucidated

Anatomically, the cornea is composed of five layers (Fig. 132) which are from without inwards:

1. Epithelium.
2. Bowman's membrane.
3. Substantia propria.

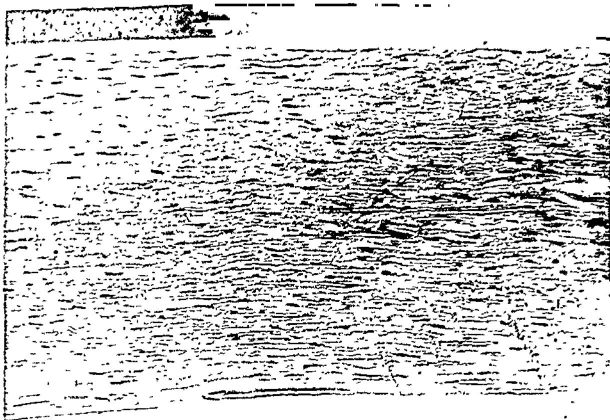


Fig. 132. Sections of the cornea showing the five layers. Note the lamellar structure of the stroma. Haematoxylin and eosin ×190.

4 Descemet's membrane
5 Endothelium

The epithelium is stratified in nature and consists of five or six layers of cells. It is *continuous with the conjunctiva*, and is of a thickness of 50-100 microns or one-fifth to one-tenth of the total thickness of the cornea.

The corneal epithelium has remarkable powers of regeneration. If the whole corneal epithelium is removed, for example with absolute alcohol, the denuded area will be almost completely re-epithelialized in 24-48 hours.

Bowman's membrane is without any cellular structure, as far as is known. The epithelium separates readily from it, but it is *closely connected with the underlying superficial stroma* of the substantia propria. It possesses no powers of regeneration.

The substantia propria, which constitutes about 90 per cent of the substance of the cornea, consists of fibrils of connective tissue and cells. *The minute bundles of fibrils* are so applied to one another (Fig. 132) as to constitute lamellae numbering 50-60 which run the entire width of the cornea, criss-crossing with each other in alternate layers and interlacing very slightly amongst each other so that they run nearly parallel to the surface. On account of this lamellar structure, a knife introduced into the cornea in making a corneal section may well run for some distance between the lamellae if it is not pushed boldly through it (Duke-Elder, 1932). The technique of lamellar keratoplasty takes advantage of this peculiarity in the arrangement of the corneal lamellae.

The fibrils consist of collagen; unlike ordinary collagen fibres, however, they lie in a matrix of mucoid material which has been shown by Woodin (1954) to be a sulphated mucopolysaccharide protein. In early embryonic life the stroma of the cornea is continuous with the sclera, and the cornea and sclera are both equally transparent, each being made up of uniform delicate fibrillae ensheathed *in mucoid*. As development proceeds and the sclera gradually becomes opaque, the fibrillae of the sclera lose their mucoid sheaths. *The fibrillae of the cornea* retain their embryonic condition and this tissue remains transparent. It is also of importance that if the corneal fibrils are destroyed they are not replaced by this peculiar type of embryonic form, but by opaque collagenous fibres of the ordinary *adult or scleral type* (Duke-Elder, 1955).

Between the lamellae lie the corneal corpuscles, flattened connective tissue cells, together with *a few wandering leucocytes*.

Descemet's membrane, unlike Bowman's membrane, can be regenerated if injured and is readily separable not only from the endothelium but also from the stroma.

The endothelium of the cornea consists of a single layer of cells 5-7 microns in thickness at the centre of the cornea, increasing to 8 or 10 microns near the periphery.

Developmentally, both Descemet's membrane and the *endothelium are continuous* with the uveal tract.

The Metabolism of the Cornea

In its normal state the water-content of the cornea is in the region of 78 per cent. Cornea is, however, able to take up water to an amazing degree, largely as a result of *imbibition by the corneal mucoid*, and if the water content increases appreciably the tissue becomes cloudy (Davson, 1949). Fundamentally, maintenance of the normal fluid balance of the cornea depends upon the intactness of the epithelium and the endothelium. Oxygen and salts enter the cornea through both of these layers of cells, while glucose and other nutrient materials are obtained through the limbal capillaries.

The metabolism of the stroma of the cornea is glycolytic and anaerobic, the lactic acid produced from the glucose in the cornea being oxidized by the epithelium. The problem of the oxygen requirement of the

cornea has been investigated by Smelser and his co-workers (Smelser, 1952; Smelser and Ozanics, 1953; Smelser and Chen, 1955). Smelser found that if pigs were fitted with contact lenses the epithelium became swollen, the glycogen granules disappearing and the lactic acid content rising. However, if a bubble of oxygen was imprisoned under the contact lens, the cornea remained in a normal state.

"There is, thus, a close symbiotic relationship between the two tissue-components, epithelium and stroma, and both must retain their vitality to maintain the metabolism of the entire structure" (Duke-Elder, 1955).

The importance of the endothelium, the inner cellular lining of the cornea, must not, however, be forgotten.

HISTORY OF CORNEAL GRAFTING

The history of corneal grafting,* or *keratoplasty* as it is sometimes called, dates from the beginning of the 19th century when Karl Himly (1813) of Vienna suggested that opacity of the cornea might be treated by replacing part of the scarred cornea with a transplant of healthy cornea taken from an animal. It was not until 1818, however, that this suggestion was put into practice by his pupil, Franz Reisinger, using experimental transplants from rabbits, pigs and dogs to humans. Most of the eyes were lost from secondary infection, but he succeeded (1824) in a further experiment in replacing the cornea of one animal with that of another, one half of the graft being transparent at the end of twenty days.

Homoplasty

It is now known that corneal transplants, if they are to remain clear, must be homologous. The first successful transplantation of this type was performed as early as 1835 by S. L. Bigger (1837). While a prisoner of a nomadic tribe of Arabs, Bigger operated upon a pet gazelle which had lost one eye, and the sight of the other from a corneal wound. The cornea was taken from another animal of the same species brought in wounded but not quite dead, adhesion took place and ten days after the operation the animal gave unequivocal signs of vision and the upper part of the transplanted cornea remained perfectly transparent."

Over 40 years were to elapse before the real significance of this work (that to be successful the transplant must be homologous) was appreciated, and in the interval attention was focussed first on heterologous grafts and then on artificial implants for embedding in the cornea.

Heteroplasty and Artificial Implants

In 1844, Kissam transplanted a portion of the cornea of a pig into the opaque cornea of a man. In this case, as in all further experiments with transplants of this nature, although the graft might "take," it invariably went opaque.

Because of the failure of heterologous grafts, workers such as Nussbaum (1853) tried the effect of artificial implants such as small glass buttons, but again without success.

Return to Homoplasty

During the latter half of the 19th century workers in different parts of the world began to be seized with the idea that to be successful the graft must be homologous. Henry Power of London, who commenced experiments on grafting in dogs, cats, rabbits and humans in 1872, concluded (1878) that, to

* A useful bibliography of corneal transplantation has been compiled by Maumenee (1954, 1955a).

remain even temporarily transparent a corneal graft must be homoplastic in nature. Sellerbach (1878) was also similarly convinced and he used the cornea of a foetus for donor material. This was followed by a report by Wolfe (1880), Surgeon to the Glasgow Ophthalmic Institution, of a full thickness homograft performed in man, the donor cornea being obtained from a freshly enucleated eye. The graft remained only partially transparent but the patient recovered a useful degree of vision.

With the discovery of ether (1846) and chloroform (1847) and the publication by Lister (1867a, b) of his classical work on antiseptic principles the stage was set for the development of the technique of corneal transplantation as we know it today.

Developments in Technique

Developments in the operative technique of keratoplasty were stimulated by the work of two men—von Hippel (1888) and Zirm (1906).

In 1888 von Hippel, fearing to open the anterior chamber because of the risk of introducing infection, performed the first lamellar (i.e. partial thickness) graft. He devised a clockwork trephine, guarded so as to penetrate the cornea only to a given depth, and his successful results gave a renewed impetus to interest in corneal grafting.

In 1906 Zirm reported a successful case of a full thickness graft and he was the first to suggest the use of overlying sutures to hold the graft in position, but another 15 years were to elapse before the work of Elschnig (1922, 1930) aroused widespread interest in full thickness grafting.

Elschnig may be said to be the father of full thickness grafting just as von Hippel was of lamellar grafting.

In the meantime Magitot (1911) had shown that a donor cornea could be kept for over three weeks in haemolysed blood at [illegible] C, and Filatov (1934) was to make use of this procedure for storing cadaver grafts.

During more recent times the technique of keratoplasty has reached nearer to perfection as a result of the work of many surgeons including Amsler, Arruga, Castroviejo, Franceschetti, Leigh, Townley Paton, Paufique, Rycroft, Sourdille and Tudor Thomas, to mention only a few.

The great development of keratoplasty *in the United States led to the necessity of* providing for storage of donor material in one centre, and the first Eye Bank was established in New York by Townley Paton in 1945.

The future history of corneal grafting will be concerned with the question of how donor material can best be preserved, and with the reaction of the host to a corneal homograft; a true understanding of the latter will guide the surgeon towards still greater perfection of operative technique.

THE BIOLOGY OF CORNEAL TRANSPLANTS

The two outstanding facts about corneal homografts which demand biological explanations are firstly that they so often remain clear indefinitely, and secondly that occasionally a graft which has remained clear for periods of up to several weeks becomes opaque.

It is clear from the evidence presented in earlier chapters (*see p. 116*) that a homograft which is isolated from host cells will behave like an autograft, and it seems likely that, as Billingham and Boswell (1953) have suggested, a corneal homograft is effectively isolated so long as the graft itself and the host cornea around it remain avascular.*

*Bacsich and Wyburn (19[illegible]) have attributed the special properties of corneal homografts to the high mucopolysaccharide content of cornea. There is, however, abundant evidence that heterotopic corneal homografts in the subcutaneous tissue and other sites where they readily become vascularized are soon destroyed. It would seem therefore that the mucopolysaccharide content is not of decisive importance except in so far as it is associated with the fact that cornea is normally avascular.

Moreover, as Duke-Elder (1955) has emphasized, a fibrous barrier is formed at the junction of host and graft which is almost *impervious to* blood vessels and nerves, and therefore maintains the isolation of the graft even when vessels have entered the host cornea.†

Clouding of a previously clear graft—the *maladie du greffon* of the French ophthalmologists (Paufique, Sourdille and Offret, 1948)—can reasonably be attributed to a breach in the barrier allowing a homograft reaction to develop and take its normal course.

Further light on the question has been provided by the experimental work of Maumenee (Maumenee, 1951, 1955b; Mueller and Maumenee, 1951; *see* also Paton, 1955a). He showed that corneal homografts in rabbits, which normally remained clear in about 50 per cent of recipients, became cloudy if the recipient was given a homograft of skin from the same donor 2-6 weeks after the corneal transplantation. On the other hand a skin homograft from another donor seldom caused clouding, and skin from the same donor transplanted 6 weeks or more after the cornea never did.

These findings suggest that corneal homografts—probably on account of their isolation—are normally not effectively antigenic, but are nevertheless for some weeks liable to invasion and destruction in immunized hosts. Thereafter they become more or less invulnerable, and the most likely explanation would seem to be that by this time an effective barrier has formed between host and graft—for this, in the light of observations on grafts in diffusion chambers, should afford adequate protection even in immunized hosts. Alternatively the cells of the graft may have been systematically replaced by host cells as Katzin (1950) suggested, or some form of adaptation (p. 96) may have taken place.

†In lamellar keratoplasty (p. 408) the usual fibrous ring is formed at the circumference of the graft but not between the deep surface of the graft and the underlying host tissue. This is because in lamellar grafting the splitting of the lamellae has been in a natural plane of cleavage—the lamellae have not been cut across—and no tissue reaction of repair has been excited.

CORNEAL GRAFTING IN PRESENT DAY SURGERY

INDICATIONS AND CONTRA-INDICATIONS

Corneal grafting is performed for one of two reasons: (1) to improve visual acuity, in cases of corneal scarring, or gross abnormality of the curvature of the cornea as in keratoconus, and (2) for the treatment of active corneal disease (therapeutic grafting).

General Considerations

Before corneal grafting is undertaken in any particular case, certain criteria must be established and one must be sure that the scarring of the cornea, or the recurrent keratitis, is not complicated by other factors which are contra-indications to operation:

1. The function of the retina and optic nerve must be such as to give a good response to the test for projection of light.

2. Corneal grafting, if performed in an attempt to improve visual acuity, should certainly not be considered unless the distant vision is less than 6/60 (20/200) and the near vision is less than N8. Townley Paton (1955b) considers that a penetrating (i.e. full thickness) graft on one eye is rarely justified if the patient has good vision in the other eye.

3. Cases of corneal scarring resulting from ulcerative keratitis in infancy are almost certain to be complicated by amblyopia "ex anopsia" and are, therefore, unsuitable for operative treatment.

4 If the eye exhibits raised intraocular tension this must be reduced to normal limits before corneal transplantation can be successfully undertaken

5 If it is known that a cataract exists in addition to the corneal opacity the corneal transplantation must be done first following which a normal extraction of the lens can be performed A full thickness transplantation performed in an aphakic eye is doomed to failure

6 Any infection of the lacrimal passages must be treated and cured before operating on the cornea

Corneal Transplantation to Improve Visual Acuity

Cases suitable for corneal grafting may be divided into three groups

1 *The most favourable*

(1) Central corneal scars with healthy surrounding cornea and no vascularization except perhaps ghost vessels In this category may be placed certain cases of interstitial keratitis and scarring from disciform keratitis

(2) Cases of keratoconus (Fig 133 and colour plate E) in which contact glasses cannot be tolerated and particularly in those in which the apex of the cone is commencing to break down

(3) Cases of Groenouw s corneal dystrophy (Fig 134 and colour plate D) which typically are ideal for lamellar keratoplasty

2 *Those in which the results are variable*

(1) In acne rosacea keratitis the results of corneal transplantation are variable Excellent results may however be obtained in cases with little vascularization or in those in which this has been appreciably lessened by preliminary beta therapy There is always however a danger in this disease of an exacerbation of the keratitis affecting the graft itself at a later date (colour plate F G H)

(2) Grafting in cases of scarring from herpetic or metaherpetic keratitis is successful in most cases but again there is the possibility of a recurrence of the original lesion in the transplant

3 *Those in which the results are unfavourable*

In this group may be placed those cases in which there is heavy vascularization of the cornea or in which so little of the cornea is free from scar tissue that the graft cannot hope to remain viable

Cases of corneal scarring in aphakic eyes are as has already been stressed totally unsuitable for keratoplasty



Fig 133 Keratoconus

Therapeutic Grafts

Filatov (1937) was the first to recognize that the cornea adjacent to a graft might become relatively clear even although the transplant itself might become opaque and there is no doubt that this trophic effect of the graft can be of great value in the treatment of certain recurrent inflammatory or degenerative lesions of the cornea

The three main indications for a therapeutic graft are

1 Indolent corneal abscesses when the affected area can be totally excised and replaced by a graft

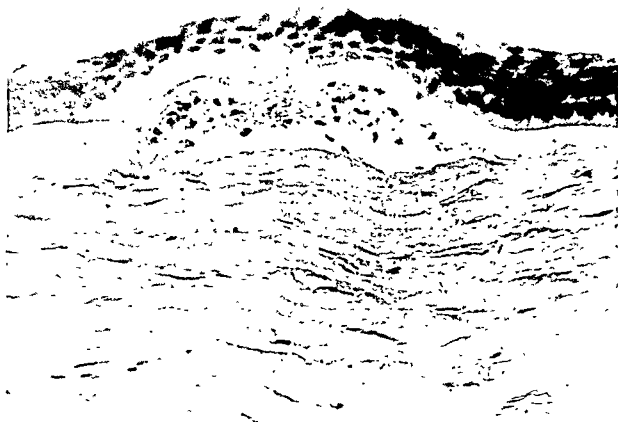


Fig 134 Groenouw's dystrophy Section of the cornea showing the superficial position of the lesions ideally suitable for lamellar grafting Haematoxylin and eosin. ×375.

2 Perforating ulcers of the cornea which have resulted in a descemetocele.

3 Certain cases of herpetic or metaherpetic keratitis

Clinical Examples

Favourable Cases

R. H. Male; aged 24 years; Bilateral Keratoconus with degenerative changes in the centres of both corneae. High degree of myopia and irregular astigmatism in both eyes (R −15.0 sphere, −6.0 cylinder; L: −21 0 sphere, −8 0 cylinder). Had become intolerant to contact lenses. Six millimetre penetrating graft inserted in left cornea (colour plate E) Final visual acuity 6/6 and N5, when corrected for a moderate degree of mixed astigmatism (−2 0 sphere, +3.5 cylinder)

E. A. Female; aged 41 years; Acne Rosacea Keratitis. Corneal scarring both eyes from old acne rosacea keratitis, mainly affecting the central portions of the cornea (colour plate F). Visual acuity reduced to less than 6/60. Four millimetre penetrating graft inserted in each cornea (colour plate G) Final visual acuity 6/9 and N5 in each eye with contact lenses to correct a moderately high degree of astigmatism.

Less Favourable Cases

W. P. Male; aged 49 years; Acne Rosacea Keratitis. Vascularized corneal scarring in both eyes with visual acuity reduced to "barely 6/60." Four millimetre penetrating graft inserted in left cornea, after which it was seen that he also had a considerable degree of cataract as well as posterior synechiae from previous attacks of iritis

Cataract removed from left eye four months after insertion of the corneal transplant. Final visual acuity 6/18 and N5 when corrected. Tendency to develop slight but

recurrent attacks of superficial keratitis in the graft controlled by *cortisone drops*

W. H. *Female, aged 12 years, Acne Rosacea keratitis. Vascularized corneal scarring in both eyes with visual acuity less than 6/60.* Six millimetre penetrating graft inserted in the left cornea; visual acuity restored to 6/12 and N5 when corrected for a moderate degree of hypermetropia and astigmatism. Transplant remained clear for six months, at the end of which time she developed an area of ulcerative keratitis in the graft (colour plate II).

TYPES OF GRAFT USED

Only homografts are used. There is virtually no opportunity for autografting, and heterografts, despite some claims to the contrary (Golovine, 1955), are unreliable.

Corneal homografts may be divided into two categories:

1. Full thickness grafts.
2. Lamellar (partial thickness) grafts, in which the cornea of the host is removed to a variable depth leaving the endothelium, Descemet's membrane and some of the deeper lamellae intact.

A successful full thickness graft should give the best visual result but, as Rycroft (1955) has emphasized, this applies only to successful cases, and complications such as anterior synechiae, secondary glaucoma, riding of the graft with a resultant high degree of astigmatism or opacification of the transplant are frequent. These complications are avoided in lamellar keratoplasty, which is certainly the operation of choice when the corneal opacity does not affect the deeper layers of the stroma.

As Duke-Elder (1955) has stressed, there are three essentials for successful keratoplasty:

1. There must be good apposition between the graft and host so that the endothelium and the epithelium may be rapidly reconstituted.
2. The graft must be in apposition with reasonably healthy cornea at some point in its circumference if it is to remain transparent.
3. Blood vessels must not invade the graft to any appreciable extent.

Attempts to find ways in which these objectives may best be achieved have led to many modifications in operative technique.

TECHNICAL CONSIDERATIONS

Methods Designed to Achieve Good Apposition Between Graft and Host

When the surface of the cornea is so uneven that accurate edge to edge apposition of the graft and the host cornea would be impossible, a lamellar graft may first be inserted (Fig. 135) so as to render the cornea of even thickness, and this operation is followed at a later date by a penetrating graft to seek to restore good visual acuity.

One of the greatest problems in full thick

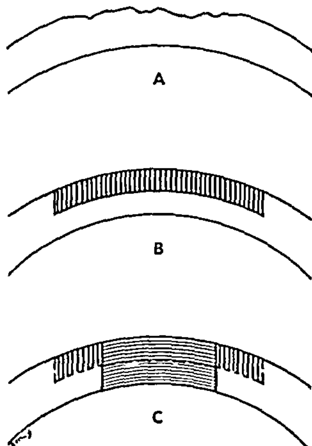
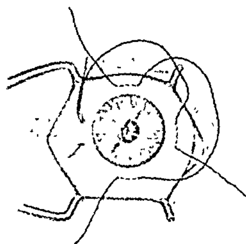
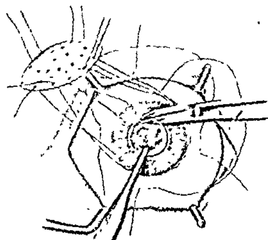
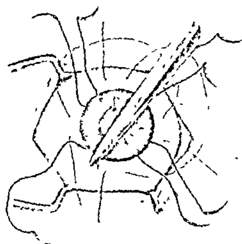
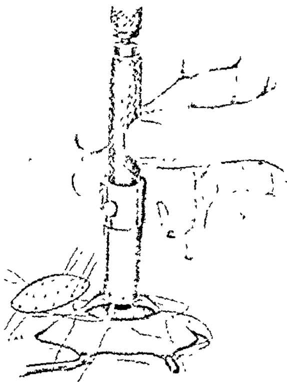


Fig. 135. Building up an uneven cornea (A) with a lamellar graft (B) before inserting a penetrating graft (C).









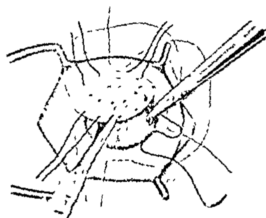
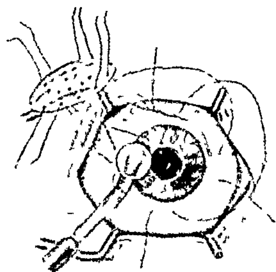
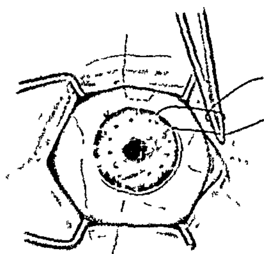


Fig 156 Technique of full thickness corneal grafting A Insertion of scleral sutures which will ultimately be used to hold the corneal splint in firm apposition to the cornea B Insertion of sutures to attach the splint to the cornea C Needles of corneal sutures being passed eye-foremost through the corneal splint D Splint laid aside to allow the cornea to be trephined E Completing removal of the disc of cornea with scissors

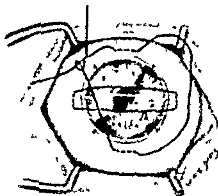
ness keratoplasty is how best to hold the graft in accurate apposition with the host *cornea*. Some surgeons prefer edge to edge *suturing, others use overlying sutures* and others again prefer the use of some form of corneal splint. Paton (1952) suggested the use of small perforated plastic strips, through which the sutures are threaded, to protect the graft from trauma by the overlying sutures, and Philps and Fincham (1952) devised a small plastic splint having twelve small holes around its edge to allow it to be *stitched to the cornea*. Sometimes one or two of the stitches cut out of the *unhealthy cornea in which the transplant is* inserted, but this complication can be avoided *by placing two scleral stitches* which, at the end of the operation, can be tied across the splint to hold it firmly in place. The stages of this method of *full* thickness keratoplasty are depicted in Figures 136 and 137.



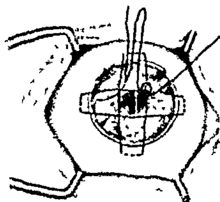
A



B



C





D

Fig. 137. Technique of full thickness corneal grafting (continued). A. Inserting the transplant. B. Corneal splint being secured in position. C. Scleral sutures being tied over the splint. D. A final suture being inserted to hold the scleral sutures firmly in place over the corneal splint.

Penetrating Keratoplasty (Modified Philps Technique)

Removal of the Transplant from the Donor Eye

The donor eye is washed in sterile saline and the cornea is then removed by inserting a Graefe knife across the anterior chamber, the incision being made as in the case of a cataract operation, and completing the separation of the cornea with scissors The excised cornea is placed on a piece of rubber or on a paraffin-wax base with its endothelial side uppermost, and the transplant is cut by firm downward pressure of a corneal trephine The graft is then washed with saline to free it from traces of iris pigment.

Insertion of the Transplant in the Recipient Eye

The operation may be performed under local or general anaesthesia

Placing the Scleral Sutures. A needle threaded with 6/0 black silk is inserted through the bulbar conjunctiva at 3 o'clock, 3 mm from the limbus, and made to take a firm bite of the sclera, the suture is then carried across the cornea and the needle is passed through the sclera at 9 o'clock in a similar fashion (Fig 136A). A second scleral suture is inserted in the vertical meridian. The sutures are laid aside so as to leave the cornea exposed for the insertion of stitches which are to attach the splint to the cornea.

Placing the Corneal Stitches. The corneal sutures, four in number, consist of fine Kalt silk, each suture being armed at each end with a fine Grieshaber needle. The needles are inserted in the cornea as in Figure 136B, taking as firm a bite as possible. The needles are then passed through the appropriate holes in the plastic splint (Fig. 136C) with the eye of the needle foremost to avoid damaging the point of the needle. The corneal splint, so threaded, is then laid aside leaving the cornea clear for trephining (Fig. 136D).

Cutting the Disc of Cornea. In many cases, the aqueous will escape before the cornea has been completely trephined and in this event the removal of the disc of cornea is completed with scissors (Fig. 136E).

Insertion of the Transplant and the Fixation of the Plastic Splint. The transplant is inserted as in Figure 137A, the plastic splint is placed in position and its fixation sutures are tied (Fig. 137B); the scleral stitches are then tied across the shield (Fig 137C) and a third stitch is inserted (Fig. 137D) to hold the overlying scleral sutures firmly in position.

The sutures are removed under general anaesthesia 14 days after the operation.

Lamellar Keratoplasty

In the case of lamellar keratoplasty, no splints are required since the anterior chamber has not been opened. The graft is secured in place by two or three direct sutures, using fine Kalt silk and the finest of Grieshaber needles. Overlying sutures are also inserted and the graft is best protected by placing a piece of egg-membrane between the surface of the transplant and the overlying sutures, as suggested by Paufique.

The various stages in the operation of lamellar keratoplasty (Paufique's technique) are depicted in Figure 138. Sutures are inserted either in the sclera near the limbus, or in the cornea itself as in Figure 138A. The cornea is trephined to the decided depth, usually one-half to two-thirds of the corneal thickness, depending upon the depth of the corneal scarring. The dissection of the opaque disc of cornea is begun with Paufique's angled knife (Fig. 138B, C) a fine silk suture is then inserted through the free edge of the disc (Fig. 138D), and dissection of the disc is continued with

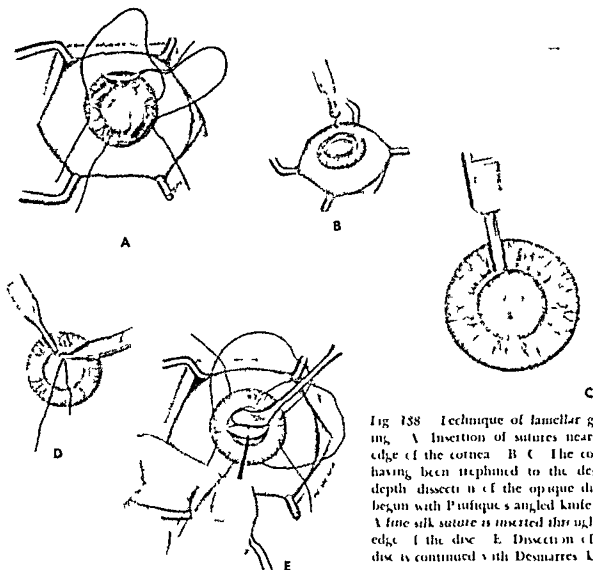


Fig. 138. Technique of lamellar grafting. A, Insertion of sutures near the edge of the cornea. B, C, The cornea having been trephined to the desired depth, dissection of the opaque disc is begun with Paufique's angled knife. D, A fine silk suture is inserted through the edge of the disc. E, Dissection of the disc is continued with Desmarres' knife.

Desmarres' knife (Fig. 138E). The transplant may be dissected from the donor cornea in the same way; alternatively, after the cornea has been trephined and the plane of cleavage defined with the angled knife, the disc may be dissected with a safety razor blade, in which case the dissection must start in the layers of cornea peripheral to the disc.

The corneal transplant is then inserted in the bed which has been prepared for it in the host cornea and covered with a piece of egg membrane, and the pre-placed sutures are tied over the membrane to hold the graft firmly in place.

Methods Designed to Prevent Vascularization of the Transplant

The local use of cortisone and pre-operative treatment of the cornea with beta rays reduce the risk of vascularization to only a relatively slight extent.

It was noticed by Leigh (1955) that, if a lamellar graft was inserted in a cornea with much superficial vascularization, while the graft usually remained clear, a dense vascular network might develop between the recipient cornea and the graft. Leigh, therefore, devised an ingenious method of preventing this vascular intrusion. The opera-

tion (Fig 139) is done in three stages. At the first operation, a central lamellar graft of, say, 6 mm. diameter is inserted. After a period of 6 to 9 months, an annular graft is inserted, leaving a ridge of recipient cornea between the inside of the annular graft and the outer edge of the central graft Six months later, if both the annular graft and the central lamellar graft are relatively clear, a central full-thickness transplant may be inserted within the area of the original central lamellar graft.

Leigh found that new vessels growing into the cornea tended to pass under the annular graft and then over the ridge of recipient cornea, by which time a barrier of scar tissue should have formed between the full-thickness transplant and the host cornea

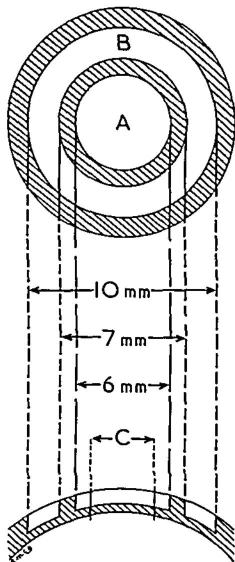


Fig 139 Diagram illustrating Leigh's annular lamellar graft A Site of central lamellar graft B Site of annular lamellar graft inserted several months later C Site of final penetrating graft (Reproduced from the *British Journal of Ophthalmology* by courtesy of the publishers and Mr A G Leigh)

STORAGE OF CORNEA

It is generally agreed that the donor eye should be removed from the donor as soon as possible after death

In America, the Eye-Bank for Sight-Restoration, in New York, recommends removal of the eye within one hour of death, although it is pointed out that, if the eyelids are carefully closed and the body refrigerated, an interval of up to 5 hours is permissible (Paton, 1955b). In other countries a longer interval is often regarded as allowable, but all would agree that eyes enucleated more than 15 hours after death are unsuitable for corneal transplantation.

It is best to preserve the whole eye in sterile liquid paraffin at a temperature of 3-5°C. and to remove the graft when required. A suture should be passed through the stump of the severed optic nerve to allow the eye to be suspended in a container without the cornea coming into contact with the bottom or sides of the vessel.

As has been mentioned in Chapter 9 attempts have been made to store cornea for longer periods in the frozen state. Grafts of cornea frozen without special pre-treatment have proved unsatisfactory, but encouraging results have been obtained by Eastcott

Cross, Leigh and North (1954) with cornea frozen after pre-treatment with glycerol

As might be expected, freeze-dried cornea has not proved satisfactory (Katzin, 1947).

THE FUTURE OF KERATOPLASTY

While, as Rycroft has emphasized, full-thickness grafting should theoretically, and does in successful cases, give the best visual result, the potential dangers and complications of this operation are often forgotten

As we have seen, the two great practical difficulties are, firstly, how to obtain accurate edge-to-edge apposition between the transplant and the host cornea and, secondly, how best to prevent vascularization of the graft

From the point of view of further perfection of operative technique, the future would seem to lie in a combination of lamellar and full-thickness grafting, a preliminary lamellar graft being used to build up a cornea of uniform thickness or to improve the metabolism of the devitalized cornea and to lessen the tendency to vascularization, and a small (4 mm) central full-thickness graft being inserted at a later date, to restore good visual acuity This would avoid the dangers (such as the formation of anterior synechiae) which are so likely to complicate the use of larger full-thickness transplants

Further developments will be dependent upon a better understanding of the metabolism of the cornea and of the immunological relationship between the host and the graft

CHAPTER 21

Transplantation of Blood Vessels

HISTORY

In the history of blood vessel transplantation there have been two periods of great activity, separated by an interval of more than 25 years during which there was very little progress in this field of surgery.

THE FIRST PERIOD—PRIOR TO 1914*

During the period prior to the outbreak of the first world war there were important developments in four directions. In the first place, reliable methods of repairing and anastomosing blood vessels were developed. Secondly, it was shown in experimental animals that orthotopic autologous, homologous and sometimes heterologous, arterial transplants were capable of functioning for long periods of time, and that venous transplants could also be used successfully to replace excised arterial segments. Thirdly, considerable progress was made in the search for satisfactory methods of storage, and finally, blood vessel transplants were used clinically in a few cases.

During this period the greatest individual contribution on the experimental side was that of Alexis Carrel, whose pioneer work was fittingly recognized by the award of a Nobel prize in 1912.

Repair and Anastomosis of Blood Vessels

As long ago as 1759 an English surgeon, Hallowel, repaired a wound in the brachial artery of a patient by placing a pin through the lips of the wound and passing a thread around it. The patient survived but there is no evidence to show whether or not the vessel remained patent, and attempts by Asman (1773) to close experimental arterial wounds in dogs by the same method proved unsuccessful.

There were no further developments in reconstructive vascular surgery until the beginning of the Listerian era when Gluck (1882), after trying unsuccessfully to repair experimental arterial wounds by suture, succeeded in doing so by means of a small ivory clamp which he applied and left *in situ*. The same year Schede (1882) successfully repaired a wound in a vein by suture, in 1886 Postempski, according to Höpfner (1903), repaired a wound in the femoral artery; and soon afterwards Jassinowsky (1889, 1891) proved conclusively in dogs, horses and calves that wounds of large arteries could be repaired by suture with preservation of the lumen of the vessel. Subsequently, Jassinowsky's work was confirmed and extended by Murphy (1897), Silberberg (1899) and Dörfler (1899) in animals, and by many surgeons in man (for review *see* Schmitz, 1903; Hopfner, 1903).

The problem of re-uniting the ends of completely divided vessel was tackled in three ways: by suturing the ends together, by invaginating one end into the other, and by using prostheses of various kinds. Whatever the method it is of course necessary to

*For the history up to 1907 I have drawn largely on the excellent review by Watts (1907a)

occlude the vessel above and below until the anastomosis is complete, and for this purpose small spring clamps known as *serrefines* or bulldog clamps were designed (see Fig. 12A).

The first successful anastomoses by suture alone were performed by von Horoch (1888) who divided and re-united the femoral and jugular veins in dogs, and by Jaboulay and Briau (1896) who, after unsuccessful experiments in dogs, succeeded in anastomosing the divided carotid artery of a donkey. Subsequently Murphy (1897), Salvia (1902), Thomaselli (1903, 1904), Jensen (1903), Amberg (1903), de Gaetano (1903) and Dorrance (1906) reported occasional instances of successful sutured arterial anastomoses in dogs and other animals, and Clermont (1901) reported a single successful suture of the inferior vena cava in a dog, but the first experiments to yield a high proportion of successful results were performed by Carrel and Guthrie (Carrel, 1902, 1905, 1906, 1907b; Carrel and Guthrie, 1906a, b; Guthrie, 1908a).

The type of suture has differed with different investigators. Jaboulay and Briau (1896) and Thomaselli (1903) used interrupted everting sutures; Clermont (1901) and Dorrance (1906) a continuous everting stitch. Carrel (1902) devised a method which was subsequently widely used, in which three traction sutures are first inserted at points equidistant on the vessel circumference, and then a simple continuous stitch is inserted between each pair of guide sutures in turn. This technique has since been modified in several ways, notably by using mattress traction sutures, by using an everting continuous stitch instead of a simple over-and-over stitch, by using either more (Fromm, 1908; Shumacker, 1948) or fewer (Eastcott, 1953) traction sutures, and by dispensing with traction sutures entirely. Some of these procedures are illustrated in Figures 13-15. Another type of anastomosis in which tantalum staples are inserted by means of a special machine will be considered later (p. 421).

Anastomosis by invagination without using a prosthesis was introduced by Murphy (1897) on the ground that it gave a stronger union than simple suture. The method proved successful in a few experiments and was used clinically by Murphy himself, who successfully re-united the ends of a divided femoral artery in a patient who had sustained a gunshot wound in the groin, and by Djemil (1896), Krause (1900), Kummell (1900) and Brougham (1906). It was given further trial by Dorfler (1899) in experiments in dogs, but without much success, and was abandoned.

Anastomosis by means of a prosthesis was tried first by Abbe (1894), who re-united the divided femoral artery of a dog over a thin glass tube; as might be expected, however, the vessel thrombosed. Abbe united the divided aorta of a cat in the same way and, because the animal survived, assumed unjustifiably that the anastomosis was functioning. Subsequently de Gaetano (1903) used an intravascular glass prosthesis to facilitate anastomosis by suture, but wisely removed it when the suture was nearly complete.

Nitze (1897) devised an extravascular prosthesis of ivory, and soon afterwards Payr (1900, 1904) published a description of a similar prosthesis made of magnesium, a substance which is slowly absorbed in the body. To anastomose an artery by Payr's method the proximal end of the vessel was passed through the tubular prosthesis, everted over it to form a cuff, and fixed with a ligature; the distal end was then drawn over the everted cuff and fastened with another ligature. To anastomose a vein the process was similar except that the distal end was invaginated into the proximal instead of *vice versa*. Despite some good results reported by Payr himself and also by Hopfner (1903), this method of anastomosis proved unsatisfactory and was soon abandoned in

favour of anastomosis by suture. It seems likely that the poor results obtained with Payr's prosthesis were due in part to the fact that magnesium in the process of absorption is converted to a strongly alkaline salt which provokes a violent tissue reaction.

Other forms of prosthesis which have been used in recent years for blood vessel anastomosis will be considered later (p. 432).

Transplantation in Experimental Animals

Transplantation of arterial segments was first attempted by Jaboulay and Briau (1896), but thrombosis occurred in every case. The transplants were autologous and the anastomoses were sutured. In 1903 Exner made vein to vein, artery to artery, and vein to artery transplants in dogs using non-sutured anastomoses made with Payr's tubes, but once again thrombosis occurred in every case. In the same year, however, the first successful arterial transplantation was performed by Hopfner (1903), who replaced an excised segment of the carotid artery of a dog with part of the femoral artery of another dog and found that the transplant was pulsating when inspected 45 days later. Hopfner, like Exner, made his anastomoses with Payr's tubes.

Successful arterial transplantation using sutured anastomoses was first reported by Alexis Carrel (1905, 1906, 1908a). Carrel appeared to believe that successful arterial homotransplants survived permanently; as we have seen (p. 42), however, it has now been shown that, whereas autologous vessel transplants may survive to a large extent, homologous ones die and are, at least in part, replaced by host tissue.

Heterotransplantation of blood vessels was first performed by Hopfner (1903), who transplanted segments of aorta from rabbits and cats into the femoral artery of the dog using non-sutured anastomoses made with Payr's tubes, but haemorrhage or thrombosis occurred in every case. The first successful instances of heterotransplantation were reported by Carrel (1907a), Stich, Makkas and Dowman (1907), Guthrie (1907a), and Ward (1908). These investigators all used sutured anastomoses. Carrel transplanted dog carotid to the aorta of the cat. Stich, Makkas and Dowman, and Guthrie transplanted rabbit and cat aorta, and segments of human arteries from freshly amputated limbs, to dogs. Ward made a careful histological examination of segments of rabbit aorta which had been transplanted to the carotid of the dog ten weeks previously. The transplants, which were functioning perfectly, were moderately dilated and the wall was slightly thinned. The intima had disappeared and been replaced by a layer of fibrin, the muscle had been largely replaced by connective tissue in which numerous fine haemorrhages could be seen, and all elastic tissue had disappeared. Ward concluded that arterial heterotransplants were replaced by host tissue but might nevertheless continue to function, and this has been confirmed by the more extensive experiments of Stich and Zoeppritz (1909), Klotz, Permar and Guthrie (1923), and other workers.

Arteriovenous anastomosis was performed successfully by Gluck (1898), and Carrel and Morel (1902a, b), who united the carotid artery and external jugular vein in dogs by sutured anastomosis, but attempts by Exner (1903) and Höpfner (1903) to perform the same operation with Payr's prosthesis were unsuccessful. The technique of arteriovenous anastomosis was perfected by Carrel and Guthrie (1905a, 1906a, b).

Autotransplantation of a segment of vein to restore the continuity of a divided artery was attempted by Gluck (1898), Exner (1903) and Hopfner (1903), but thrombosis occurred in every case. The operation was performed successfully in experimental animals for the first time by Carrel and Guthrie (1906b), who transplanted segments of external jugular vein to the carotid artery,

and of femoral vein to the femoral artery in dogs and subsequently by Watts (1907a, b). Stich and his colleagues (Stich, Makkas and Dowman, 1907; Stich and Zoeppritz, 1909) and Borst and Enderlen (1909a, 1910). Carrel and Guthrie described the transplantation as *complete* when a segment of vein was completely isolated before being anastomosed to the ends of the artery and as *incomplete* when a segment of vein was transplanted to an adjacent artery without being dissected from its bed and without its tributaries being divided. They reported that the results of complete and incomplete transplantation were very similar. In both cases the transplant became dilated and its wall became greatly thickened. These changes were well marked 11 days after transplantation. Histological examination at this stage showed a great increase of fibrous and elastic tissue in the media and also in the inner part of the adventitia (*see also* Carrel, 1910d).

Methods of Storage

Carrel (1907a, 1908a, 1910c, 1912a) and Bode and Fabian (1910) experimented with homotransplants and heterotransplants of vessels stored at ice-box temperatures (0° to 1 C). Carrel reported that transplants stored for 10-11 days yielded consistently good and permanent results and preserved their gross appearance; they showed well marked histological changes however, notably loss of muscle fibres. Transplants stored for periods up to several months often yielded good functional results but showed even more marked histological changes. Bode and Fabian, however, found that transplants stored for more than 35 days usually thrombosed.

Carrel (1908a) also experimented with transplants which had been stored frozen. He found that while the immediate results were usually good, the transplants ceased to function after a few days, weeks or occasionally months.

Levin and Larkin (1907, 1909, 1910) and Guthrie (1908b) transplanted segments of vessels preserved in formalin. They reported that transplants to the aorta gave fairly good results but transplants to peripheral arteries were not satisfactory.

Clinical Trial of Blood Vessel Transplants

In 1906 Goyanes performed clinically an operation of the type described by Carrel (*v. supra*) as *incomplete* transplantation. The patient had an aneurysm of the popliteal artery which was treated by dividing the artery proximal and distal to the aneurysm and then restoring the circulation by isolating a segment of the vein and anastomosing this segment to the proximal end of the femoral artery above and to the distal end of the popliteal artery below.

Free or *complete* blood vessel transplantation was performed in a patient for the first time by Lexer in 1907, when he resected an aneurysm of the axillary artery and used an autotransplant of a segment of saphenous vein to restore continuity of the vessel. The transplant thrombosed but some years later Lexer (1912a, 1913) reported two cases in which he had successfully resected arterial aneurysms and replaced them with autologous vein grafts. Another patient in whom this operation was performed died a few days after operation but the transplant was still patent. Three further cases in which an aneurysm was successfully resected and replaced by an autologous vein graft were reported in 1913, two of them by Pringle of Glasgow and one by a German surgeon, Coenen. The specimen obtained at autopsy from one of Pringle's cases 2½ years after the operation is illustrated in Figure 140.

THE MIDDLE PERIOD—1914 TO 1939

A large series of successful reconstructive operations for traumatic aneurysm and arteriovenous fistula, in which a main artery

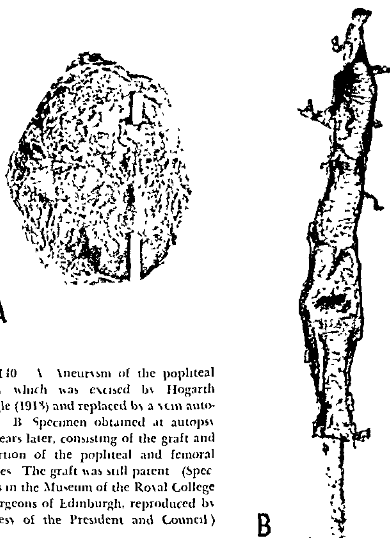


Fig 140 A Aneurysm of the popliteal artery which was excised by Hogarth Pringle (1913) and replaced by a vein autograft B Specimen obtained at autopsy 31½ years later, consisting of the graft and a portion of the popliteal and femoral arteries The graft was still patent (Specimens in the Museum of the Royal College of Surgeons of Edinburgh, reproduced by courtesy of the President and Council)

a main vein, or both, were repaired by suture, was reported in 1914 by Subbotitch from his experience in the Serbian wars. During the first world war similar reconstructive operations were performed by surgeons on both sides and, in addition, gangrene was prevented in some cases by Tuffier's (1915, 1917) technique of inserting a paraffin-coated silver cannula to restore continuity temporarily in a severed artery (Makins, 1919), there were, however, few cases in which vessel transplants were used in the treatment of recent injuries, though they were used in the definitive treatment of traumatic aneurysms by von Bonin (1915), Hotz (1915), Zahradnicky (1915), Warthmuller (1917), Allesandri (1921), Goodman (1921, 1913), Sencert (1921), Weglowski (1925), who reported 51 cases in which he used an autologous vein graft, and other surgeons. At first sight this may seem surprising but on reflection it is apparent that, under the conditions which obtained on the Western Front, blood vessel transplantation in the absence of chemotherapeutic agents and antibiotics to control infection would have been doomed to failure.

The year 1916 saw the first report from America (Bernheim, 1916) of the successful

use of a vein autotransplant in the treatment of a popliteal aneursym as we have seen however similar reports had been published previously in Germany and Great Britain

Between the wars arteriography was performed for the first time by Brooks (1924) who used a solution of *sodium iodide* as the contrast medium The procedure was further developed by Reynaldo Dos Santos* of Portugal and his colleagues who applied it *to the aorta (Dos Santos Lamas* and Caldas 1929 1931) as well as to peripheral arteries (Dos Santos 1935) and later reported on the

For many years however opinion on the propriety of these procedures was divided and many investigators stressed the dangers of both peripheral arteriography (*see* Wagner 1944 for review) and aortography (Henline and Moore 1936)

THE THIRD PERIOD—1939 ONWARDS

*Many factors have contributed to the re*naissance of vascular surgery notably the discovery first of sulphonamides and later of penicillin the development of anticoagulant therapy (p 117) initiated by the discovery of Gordon Murray (1939 1940) that in the absence of infection the incidence of thrombosis following blood vessel anastomosis could be significantly reduced by administering heparin for a day or two after operation the urgent demands of war the advances in anaesthetic technique which have so greatly facilitated operations within the chest and refinements in the technique of arteriography (*see* Learmonth 1944 Lindbom 1950) and aortography (Freeman Fullenlove Wylie and Gilfillan 1949 Wagner and Price 1950 Deterling 1952 Leriche Beaconsfield and Boely 1952 Stirling 1957)

During this period the technique of vascular anastomosis and replacement has been *further investigated* and the clinical indications for these procedures have been greatly *extended* as methods of avoiding ischaemic damage to important structures during grafting operations have been developed In addition methods of storage have been im*proved* blood vessel banks have been established in many hospitals and the behaviour of stored homotransplants and heterotransplants has been studied extensively both in experimental animals and in patients and in consequence blood vessel transplantation has been used clinically to a much greater extent than ever before Finally prostheses of many different kinds have been devised and tested and some of them have proved so successful that homografts are being used much less frequently than they were a few years ago and seem likely soon to become *obsolete*

Vascular Anastomosis

Smith (1940) devised a soluble rod made of dextrose and coated with glycerin or oil for use as a temporary intraluminal splint to facilitate blood vessel anastomosis by suture He used this technique successfully to anastomose the divided carotid artery in dogs and reported that the rod dissolved in a minute or less after the circulation was restored The method does not appear to have been used clinically

Blakemore Lord and Stefko (*1942 1943*) searching for a rapid method of restoring arterial continuity which could be used successfully in battle casualties experimented in dogs with anastomosis by means of prostheses made of vitallium The methods they *tried were as follows*

1 Anastomosis over an intraluminal vitallium tube

2 Gap in artery bridged with a vitallium tube

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*In the first paper on the subject Dos Santos *et al* (19[illegible]) reported 200 cases in which they performed [illegible] using sodium iodide as contrast [illegible] and [illegible] that there was no appreciable mortality [illegible]

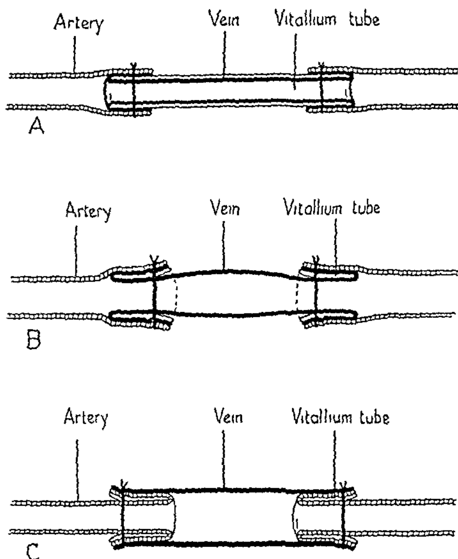


Fig 141 Three methods of using a vein graft in conjunction with the Blakemore Lord prosthesis for arterial reconstruction The figures A, B, C correspond to the third, fourth and fifth methods described in the text respectively. (Reproduced from *Surgery* by courtesy of the publishers and Dr Arthur H. Blakemore.)

3. Vein graft passed through a vitallium tube and ends everted. Graft thus mounted used to bridge gap in artery (Fig 141A).

4. Each end of vein graft passed through and everted over a vitallium tube, the central part of the vein being unsupported. Graft thus mounted used to bridge gap in artery (Fig. 141B).

5. Ends of divided artery each passed through a vitallium tube and everted; gap then bridged with a vein graft (Fig. 142C).

The first two methods were completely unsuccessful; the third was successful in only two animals out of seven. The last two methods on the other hand were regularly successful when applied to the femoral artery. No anticoagulants were used in any of these experiments.

For the most part Blakemore *et al.* used fresh vein autografts, but they were successful also with fresh homografts,* and in later

*Blakemore *et al* unfortunately use the term *heterograft* to denote a graft from one animal to another of the same species as well as a graft to a member of a

experiments Blakemore and Lord (1945a) reported good results with fresh heterografts, and in a few cases with stored grafts. The technique was subsequently modified by Swenson and Gross (1947), who experimented with absorbable prostheses made of fibrin, which, they suggested, might be used for vascular anastomosis in children, and by Oudot (1948a, b), who used prostheses made of a plastic material.

The good results obtained in experimental animals with non-sutured anastomoses encouraged clinical trial in two types of operation.

In the first place the method was used to restore continuity in divided arteries. Blakemore and Lord (1945a) obtained some good results in civilian patients, especially when anticoagulants were used; in two small series of battle casualties reported by Kirtley (1945) and Stewart (1947), however, the results were not impressive.

Secondly, non-suture anastomosis with the Blakemore-Lord prosthesis was used to establish a shunt between the portal and systemic venous systems in patients with portal hypertension. Anastomosis of the portal vein to the inferior vena cava has been widely used in experimental animals for studying the physiology and pathology of the liver ever since the operation was first performed by Eck in 1877. Attempts to perform a similar operation in man were, with a few notable exceptions (Rosenstein, 1912; Bogoras, 1913), unsuccessful (*see* Whipple, 1945, 1946a, for review), and the procedure was abandoned for many years. It was revived as the result of fruitful collaboration between A. O. Whipple and his colleagues Blakemore and Lord, who, in 1945, reported ten cases of portal hypertension, five of whom had been treated by splenorenal venous anastomosis and five by portocaval anastomosis (Whipple, 1945; Blakemore and Lord, 1945b). In all these patients the anastomosis was made by the vitallium tube non-suture technique, and Whipple (1945) has stated that he was deterred by technical difficulties from attempting the operation until this technique had been evolved. It was shown by Blalock (1945, 1947), however, that splenorenal and portocaval venous anastomosis by suture was technically possible, and it soon became apparent that it gave better results than anastomosis by the Blakemore-Lord technique.

Interest in anastomosis by prostheses therefore waned, whereas anastomosis by suture became of ever increasing importance in the rapidly expanding field of cardiovascular surgery, as methods were devised for the surgical treatment of two important congenital disorders of the heart and great vessels, namely coarctation of the aorta and Fallot's tetralogy, and as the indications for arterial grafting were extended to include aneurysm and localized occlusion of the aorta as well as of the peripheral vessels.

The surgical treatment of coarctation of the aorta stems from the discovery of Blalock and Park (1944) that the subclavian artery was able to conduct sufficient blood to maintain life in animals in which the thoracic aorta was completely occluded. They suggested treating coarctation by anastomosing the subclavian artery to the aorta distal to the stenosed area, and this method was used in one patient by Blalock and later in another, with excellent results, by Clagett (*see* Blalock, 1947); it has been replaced, however, by excision of the coarctation and anastomosis, which was performed first by Crafoord and Nylin (1945) and shortly afterwards by Gross and Hufnagel (1945).

The surgical treatment of Fallot's tetralogy was initiated by Blalock and Taussig. The principle of their operation (Blalock and Taussig, 1945, 1946; Blalock, 1946, 1947, 1948) is that the stenosis in the pulmonary artery is short-circuited by dividing a large systemic artery (usually the subcla-

different species, and do not use the term homograft at all. In describing their work the more usual terminology, adopted throughout this book, has been adhered to.

vian branch of the innominate artery) and anastomosing its central end to the side of one of the two pulmonary arteries. An alternative procedure designed by Potts and his colleagues (Potts, Smith and Gibson, 1946; Potts and Gibson, 1948), which achieves much the same result, is to make a side-to-side anastomosis between the aorta and the pulmonary artery. These procedures deal with one set of anomalies by creating another, and they are now being replaced by direct surgical attack on the pulmonary stenosis. Attempts to deal with the ventricular septal defect at the same time, using cardio-pulmonary bypass, have resulted in a high mortality but Brock (1958) has suggested that this might be avoided by a two stage operation. The Blalock-Taussig operation has nevertheless been of enormous importance and, in addition to giving relief to many patients, has stimulated a vast amount of research in many parts of the world.

Blalock used a continuous everting suture

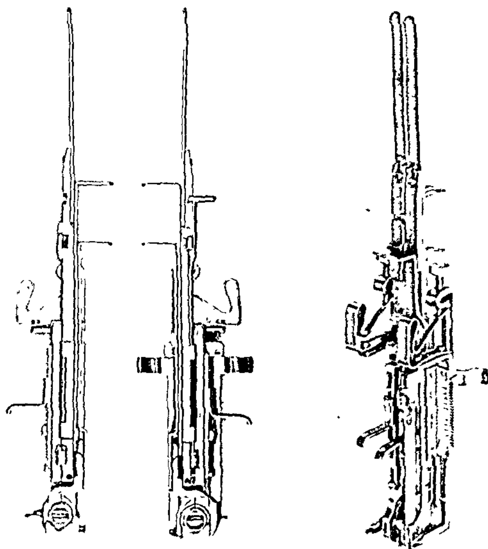


Fig 142 Androsov's machine for blood vessel anastomosis.

on the side away from the operator, which he inserted loosely with the vessels widely separated and then pulled tight as the vessels were approximated, and completed the rest of the anastomosis with a further continuous everting suture or with interrupted sutures. For anastomosis of the aorta Crafoord (1946) urged that the surgeon should aim at correct anatomical approximation of the different layers, but this suggestion was not generally accepted (Gross and Hufnagel, 1945; Shumacker and Lowenberg, 1948; Gross, 1950, 1951, and other workers) and for some years most surgeons used a continuous everting stitch. A simple over-and-over stitch has been found however to give excellent results in both experimental animals (Sako, Chisholm, Merendino and Varco, 1949) and man, and is now used by most surgeons for nearly all arterial anastomoses; some use this also for portocaval and other venous anastomoses, but others including the author prefer an everting stitch.

Holman and Hahn (1953) showed in dogs that the diameter at the site of arterial anastomosis could be increased by using a technique based on the principle of Z-plasty (p. 2..). Their procedure is rather complicated however and does not appear to have been used clinically.

Despite the failure of the Blakemore-Lord prosthesis the search for non-suture methods of anastomosis has continued and two interesting techniques have been developed by Russian workers.

The first of these employs an ingenious machine which performs the anastomosis with tantalum staples (Fig. 112). Details of the apparatus have been published in English by Androsov (1956) and Kovanov (1956). Rob (1958), who is one of the few Western surgeons with experience of the method, has found that vessels varying in size between the human common iliac and radial arteries can be anastomosed end-to-end more accurately than any surgeon can achieve by suture provided that they are normal or nearly normal, but that even a moderate degree of atherosclerosis makes it impossible to use the machine. He has concluded therefore that "the main use of the machine is in experimental surgery and in patients with normal or near normal arteries who require either a direct end-to-end anastomosis or the insertion of an autogenous vein graft by end-to-end anastomosis."

The second technique (Fig. 113), devised by Donetsky* (1956), can be used for either end-to-end or end-to-side anastomosis. The end of one vessel is everted over a thin stainless steel ring armed with radiating spikes which are sufficiently long to project about 1 mm. through the wall of the turned back portion of the vessel. The end of the second vessel, or side opening if the anastomosis is to be end-to-side, is stretched by an adjustable three-pronged dilator sufficiently to enable the end of the first vessel carrying the ring to be inserted into it. The dilator is then slackened and withdrawn and the projecting spikes of the ring engage the wall of the second vessel and thus provide a firm junction.

Whatever type of anastomosis is used the operation may be unsuccessful if the arterial clamps which are used seriously damage the vessel wall. Henson and Rob (1956) therefore performed an experiment in which they occluded the right gastro-epiploic artery in various ways for 15 minutes during the operation of partial gastrectomy and then excised the occluded segment and examined it histologically. They found that a tape tourniquet (Fig. 114) and a bulldog clamp with cloth or rubber on the blades caused little damage. Blalock's, Crafoord's and Potts' clamps (*see* Fig. 12) on the other hand caused severe damage to the media and dissection of the vessel wall.

The use of special clamps permitting some blood flow in the vessel being operated on will be considered later (p. 425).

* The author is indebted to Professor [illegible] for information about this procedure.

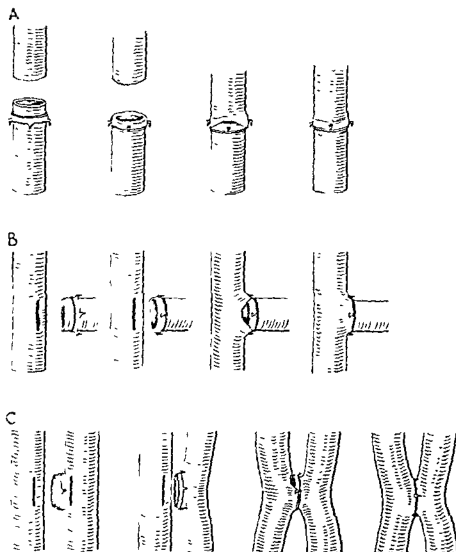


Fig 143 Blood vessel anastomosis using Donetsky's spiked ring prosthesis. A End-to-end anastomosis. B End-to-side anastomosis. C. Side-to-side anastomosis (After Donetsky.)

Types of Graft and Indications for Their Use

Fresh vein autografts continued to be used occasionally to bridge defects in peripheral arteries (see Cooke, Hughes, Jahnke and Seeley, [illegible]) and were used in two patients with Fallot's tetralogy by Johnson, Kirby, Greifenstein and Castillo (1949) to form a bridge between the subclavian and pulmonary arteries. Despite some earlier opinion to the contrary (Johnson *et al*, 1949; Johnson, Kirby, Allam and Hagan, 1951b) it was shown in animals that vein transplants in the aorta were unsatisfactory, and in particular were liable to aneurysmal dilatation (Johnson, Kirby and Hardy, 1953; Schmitz, Kanar, Sauvage, Storer and Harkins, 1953; Nyhus, Kanar, Moore, Schmitz, Sauvage, Zech and Harkins, 1955). Attempts have been made to obviate this by treating the graft with sclerosing agents (Zech, Nyhus, Kanar, Schmitz, Sauvage, Moore, Fletcher, Merendino and Harkins, 1954) or wrapping it in fascia (Sako, 1951; Griffith, Eade, Zech and Harkins, 1955) or nylon (Vargas and Deterling, 1953), but without much success.

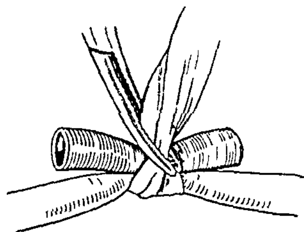


Fig. 111 Tape tourniquet A piece of rubber tubing is placed alongside the artery and both artery and tubing are encircled by the tape (Redrawn from the *British Journal of Surgery* by courtesy of the publishers and Professor C. G. Rob)

Hammer and his colleagues (Hammer, Seay, Hill and Prust 1953 1954 Hammer, Seay, Hill Prust and Campbell, 1955, Hammer, Patrick Johnston Hill and Prust, 1958) have *even* tried using pedicle grafts of small intestine denuded of mucosa as a wrapping for blood vessel grafts but it is difficult to imagine such a bizarre procedure becoming of clinical importance

Ingenious techniques have been devised for constructing large bore arterial grafts from small bore arteries and autografts of this sort have been used successfully in experimental animals (Sandblom Sandegard and Muren 1952 Hurwitt and Kantrowitz 1952a, b Sandblom, Muren, Nardén, Idbohrn Sandegard and Dahlbäck, 1953, Potts Albert and Fischer 1953) None of these techniques appear to have been used clinically however, and it seems unlikely that they ever will be

Stored arterial homografts have been used extensively in aortic and peripheral vascular surgery principally in the treatment of aneurysm coarctation of the aorta associated with a long narrowed segment or an aneurysm thrombosis at the aortic bifurcation and localized occlusion of a peripheral artery in patients with obliterative arterial disease The results will be considered in detail later (p 135) In brief aortic grafts have proved very satisfactory, but the use of grafts to replace segments of peripheral arteries in atherosclerotic patients has yielded a high proportion of failures Bypass grafting (p 142), introduced by Kunlin (Leriche and Kunlin, 1948 Kunlin 1949 Kunlin Bitry Boely, Volmic and Beaudry, 1951) and used subsequently by Crawford and DeBakey (1955), Linton and Menendez (1955) Mavor (1956) and many other surgeons, has been more successful but the long term results still leave much to be desired

Heterografts of freeze dried calf or pig vessels were used successfully in four patients* by Hufnagel, Rabil and Reed (1954) Subsequently Sautot Bost, Fouraine, Martin and Feroldi (1954) reported good results with heterotransplants of calf artery in one patient with an arterial injury and in a proportion of patients with obliterative arterial disease Further trial therefore appeared to be justified, especially—in the light of considerations of antigenicity discussed in Chapter 8—of non viable heterografts It was soon shown however that both formalin preserved and freeze dried heterografts of pig artery eventually weakened and dilated in dogs and also in man (Creech DeBakey, Self and Halpert, 1954) and were therefore unsatisfactory Confirmatory evidence was reported by Inahara Menendez, Shaw and Linton (1957) who obtained poor results with freeze dried heterografts of bovine artery transplanted to dogs

The Problem of Ischaemia During Vascular Operations

The abdominal aorta at any rate below the renal arteries, and the peripheral vessels can as a rule,† safely be occluded for an

*Portions of calf and pig aorta were joined together and used to replace the common iliac artery in one of these patients.

†There are a few exceptions which differ in different
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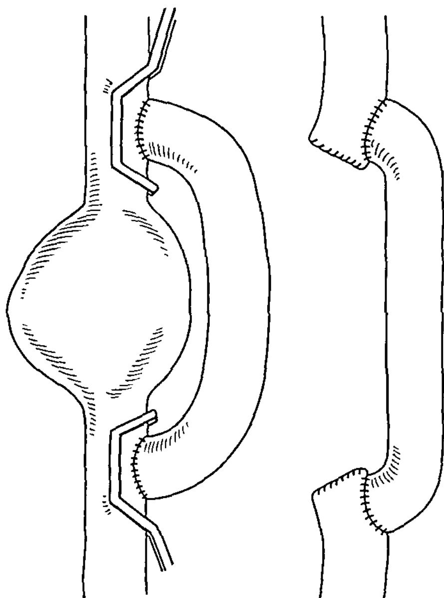


Fig 145 Diagram illustrating the technique of Mahorner and Spencer for resecting an aneurysm without interrupting the circulation through the vessel.

hour or two On the other hand, in operating on the thoracic aorta or the vena cava it may be dangerous to arrest the blood flow completely for sufficient time to complete even a single anastomosis except when there has been long standing partial obstruction, as for example in patients with coarctation. In dogs, for instance, complete occlusion of the previously unobstructed thoracic aorta for more than ten or fifteen minutes is likely to cause spastic paralysis (Carrel, 1910b; Blalock and Park, 1914).

This difficulty has been overcome in several ways: by using a special clamp which isolates a portion of the vessel without completely obstructing the blood flow; by using a temporary simple arterial shunt, or a more elaborate shunt incorporating a pump or a pump-oxygenator, to maintain an adequate

species In sheep, for example, as Borrie and Woodruff (1955) have shown, occlusion of the abdominal aorta just below the renal arteries for 30 minutes usually results in permanent paraplegia. In man the age of the patient and the presence of generalized vascular disease must be taken into consideration

circulation while the operation is in progress and by lowering the temperature of the animal or patient in order to reduce the rate of metabolism and keeping it lowered until the circulation is restored

Special Clamps

A clamp which can be used to isolate part of the aorta without completely obstructing it was described by Potts Smith and Gibson (1946) and is known as Potts aortic clamp It is illustrated in Figure 12F Similar clamps can be used on the inferior vena cava but the simpler type of clamp illustrated in Figure 12I is also satisfactory

With clamps of this sort a transplant may be connected to the host vessel by end to side anastomosis above and below the lesion (Fig 145) without interrupting the circulation blood can then be allowed to flow through the transplant while the lesion is resected This technique was devised by McWhorter and Spencer (1954a b) and was used subsequently by Adams (1955)

Temporary Simple Arterial Shunts

Temporary artificial shunts may be either intraluminal or lateral

It was shown long ago by Carrel (1912b c) that the circulation in the dog s aorta would continue for many hours through a smooth large calibre tube More recently DeCamp Spencer and Overstreet (1951) used a siliconed stainless steel tube experimentally as an intraluminal shunt during transplantation to the aorta leaving it in place until the distal anastomosis and two-thirds of the proximal anastomosis had been completed and the same principle was used to facilitate resection and replacement of aneurysms of the thoracic aorta by Lam and Aram (1951) and Johnson Kirby and Lehr (1955)

Carrel (1910b) experimented also with lateral shunts but the operation proved to be technically difficult and was abandoned In recent years however Clatworthy and Varco (1950) and Schafer and Hardin (1952) have reported favourably on the use of lateral shunts made of polyethylene in dogs and a similar technique has been used clinically by Hardin (1952) Stranahan Alley, Sewell and Knusel (1955) Cooley Mahaffey and DeBakey (1955) and other surgeons using a variety of materials including *nylon tygon ivalon* and heterologous artery grafts In these operations the proximal end of the shunt was connected to the aorta itself proximal to the site of operation or to one of its main branches and the distal end to the aorta below the site of operation

Shunt with Pump

An effective and technically not very difficult procedure is to take a shunt from the left atrium via a pump to the distal aorta or to a cannula in the femoral artery as described by Gerbode Braimbridge Osborn Hood and French (1957) and Morris Witt Cooley Moyer and DeBakey (1957) (*see* Fig 151)

Shunt with Pump-oxygenator

In 1937 John Gibbon of Philadelphia published a remarkable paper on artificial maintenance of the circulation during experimental occlusion of the pulmonary artery and thus initiated the search for an effective cardio pulmonary bypass technique using a mechanical pump-oxygenator

Today Gibbon s dream is an accomplished fact and he and his colleagues (Miller Gibbon and Gibbon 1951 Gibbon 1954) together with many others including Dennis (Dennis Spreng Nelson Karlson Nelson Thomas Eder and Varco 1951) Lillehei (Warden Read DeWall Aust Cohen Ziegler Varco and Lillehei 1955 DeWall Warden Gott Read Varco and Lillehei 1956 DeWall Warden Read Gott Ziegler Varco and Lillehei 1956 Gott DeWall Paneth Zuhdi Weirich Varco and Lillehei 1957) and Kirklin (Jones Donald Swan Harshbarger Kirklin and Wood 1955 Kirklin DuShane Patrick Donald Hetzel Harshbarger and Wood 1955 Kirklin Patrick

and Theye, 1957) in America, Bjork (1948) and Crafoord (1949) in Sweden, and Melrose (1953) in England, have helped to make it so.

Cardio-pulmonary bypass using one of the many pump-oxygenator combinations now available (for review *see* Allen, 1958) is used mainly in connexion with open heart surgery, but has also made it possible to resect an aneurysm of the ascending aorta and replace it with a graft or prosthesis, and was first used successfully for this purpose by Cooley and DeBakey (1956)

Hypothermia

In 1902 Simpson cooled monkeys which had been anaesthetized with ether and reduced their body (rectal) temperature to between 23° and 25°C A little later Simpson and Herring (1905) performed similar experiments on cats. They showed that when the temperature was reduced below a certain level no anaesthetic was necessary, and they described the phenomenon as "artificial hibernation" or "cold narcosis."

Many years later Smith and Fay (1940), in the light of a previous observation by Fay and Henny (1938) that prolonged local cooling of human tumours resulted in relief of pain and some decrease in the size of the tumour, treated cancer patients by reducing the temperature of the whole body. After giving the patient "a small anaesthetic dose of evipal to carry him through the period of discomfort while the temperature was falling from 96° to 90°F." he was placed on a rubber sheet and cooled by ice The temperature fell to 90°F in from one to two hours, and the ice was then removed; the temperature continued to fall, however, usually to 88° or 89°F. The patient was kept at this temperature for four or five days and then rewarmed. During the period of hypothermia saline and glucose was administered through a tube in the stomach, and paraldehyde was given rectally to control restlessness. Cooling was repeated three or four times at weekly intervals without ill effect and with considerable relief of pain in patients with advanced cancer.

After a lapse of nine years McQuiston (1949) drew attention to the possible advantages of hypothermia in cardiac surgery and reported the case of an infant aged 4 months whose temperature after induction of anaesthesia was reduced to 96°F. (35.6°C) with ice bags prior to a Potts type of operation for pulmonary stenosis. McQuiston stated that at least 25 children had been treated in the same way.

Mild cooling such as this, though possibly beneficial in some cases, seemed unlikely to lead to dramatic advances in cardiovascular surgery. In the following year, however, Bigelow and his associates published three papers describing experiments in dogs which suggested that cooling to considerably lower temperatures might be of decisive importance in permitting relatively prolonged occlusion of the venae cavae and thus facilitating operations on the open heart. It was shown first (Bigelow, Lindsay, Harrison, Gordon and Greenwood, 1950) that when a dog's body temperature was reduced from 38° to 20°C, the metabolic rate and oxygen consumption fell to 18 per cent of their normal values. Subsequently it was shown (Bigelow, Lindsay and Greenwood, 1950; Bigelow, Callaghan and Hopps, 1950) that dogs cooled to 20°C. would sometimes survive exclusion of the heart from the circulation for 15 minutes by clamping of the venae cavae, and cardiotomy of the left auricle. For comparison it was recalled that Templeton and Gibbon (1949) had found previously that dogs not cooled would survive clamping of the venae cavae for at most 9 minutes.

In the experiments of Bigelow *et al.* the animals were cooled by wrapping them in blankets containing coils carrying circulating refrigerant, after they had received intravenous injections of pentothal and curare

to control shivering. They were warmed either in a bath of water at 40° C or by high frequency (14 megacycles) inductive heating.* The mortality in these experiments was disturbingly high. Out of 39 dogs 19 died either during the period when the cavae were clamped or during rewarming: 14 with ventricular fibrillation and 5 with cardiac arrest. The remaining 20 were rewarmed to normal temperature but 12 of them became cyanosed and hypotensive and died a few hours later. Bigelow *et al.* attempted to deal with ventricular fibrillation by using an electric defibrillator and with cardiac arrest by using an artificial pacemaker to provide rhythmic electrical stimulation of the sino-auricular node. They were unable to assign a cause or suggest a method of treatment for the condition of collapse which developed in 12 animals after rewarming.

Cookson, Neptune and Bailey (1952a, b), by modifying the technique of Bigelow *et al.*, obtained much better results: 80 per cent of animals surviving after caval occlusion for 12 minutes and 40 per cent after caval occlusion for 30 minutes. Ventricular fibrillation was avoided by cooling to only 26° C and by injecting a short-acting adrenolytic drug, Benodaine, into the right ventricle when the clamp on the superior vena cava was released. Death at a later stage from pulmonary oedema or "shock" was largely prevented by the following measures: premedication with morphine to reduce the amount of pentothal needed for anaesthesia and atropine in large dosage to prevent the unusually copious bronchial secretion which otherwise occurs during hypothermia; intravenous administration of 50 per cent glucose; and adequate ventilation with pure oxygen during the whole period of unconsciousness.

Meanwhile in France Laborit and Huguenard (Laborit, 1950a, b, c; Laborit, 1951a; Laborit, Huguenard and Alluaume, 1952) having observed what they described as the potentiation of anaesthesia by certain derivatives of phenothiazine—in particular diethazine (Diparcol; 2987 R.P.), promethazine (Phenergan; 3277 R.P.) and chlorpromazine (Largactil; 4560 R.P.)—and having concluded that these drugs in some way blocked the autonomic nervous system,* had embarked on an extensive series of experimental and clinical investigations in which they combined administration of these and other drugs with external cooling (Laborit and Huguenard, 1951, 1952; Laborit, 1951b, c; Huguenard, 1953). Following the example of Simpson and Herring they called the procedure *artificial hibernation*, but this term has been strongly criticized by Juvenelle and his colleagues (Juvenelle, Lind and Wegelius, 1952; Juvenelle, 1954) and by Kayser and Hiebel (1952) on the ground that the phenomenon differs sharply from the natural hibernation of certain mammals in which autonomic nervous mechanisms persist even at extraordinarily low temperatures. These workers all prefer the term artificial hypothermia (*l'hypothermie generalisée provoquée*) and Juvenelle *et al.* add the qualification *deep* when the temperature is reduced below 25°C.

Various modifications in the method of cooling have been used.

Instead of surface cooling Delorme (1952) has used an external arteriovenous shunt in which blood circulates through a refrigerating system outside the body. The procedure has been criticized (Churchill-Davidson, 1954, and others) on the grounds firstly that the procedure is unnecessarily complex and necessitates interference with major blood vessels, secondly that the creation of a

*Microwave and short wave capacitance diathermy were also tried but proved less satisfactory.

*The French workers speak of the drugs as *ganglioplegique*, but it is clear that this does not imply that they act primarily or even at all on peripheral autonomic ganglia (see e.g. Laborit and Huguenard, 1951; Courvoisier, Fournel, Ducrot, Kolsky and Koetschet, 1953; Compte Beard, 1954).

large arteriovenous fistula, even temporarily, is dangerous, and thirdly that an excessive amount of haemolysis may occur, but Brock and Ross (Ross, 1954b, c; Brock and Ross, 1955) have used a pump-assisted venovenous external shunt from the superior vena cava to the inferior vena cava and found it very satisfactory (Brock, 1958). Another method, mentioned by Churchill-Davidson, McMillan, Melrose and Lynn (1953) but rejected on the ground that external cooling is simpler and safer, is to irrigate the pleural or peritoneal cavities with ice-cold water. Whatever method is used it is generally agreed that shivering must be prevented. This may be achieved by using deep anaesthesia, muscle relaxants, or the "lytic cocktail" of Laborit and his colleagues, and it has been found by Dundee, Scott and Mesham (1953) that in dogs all three methods are equally effective.

The problem of ventricular fibrillation has been dealt with in several ways. Churchill-Davidson *et al.* (1953) showed that in dogs fibrillation was likely to occur in adult animals cooled to 24° C. or lower, but not in puppies, or in older animals cooled only to between 26° and 28°C. Maguire and Merendino (1955) have confirmed the conclusion that puppies tolerate cooling to lower temperatures than adult dogs, and have shown that they succumb eventually not to ventricular fibrillation but to cardiac arrest. Swan, Zeavin, Blount and Virtue (1953), also working with dogs, found that the incidence of fibrillation was lessened if respiratory alkalosis was induced by deliberate hyperventilation. When fibrillation occurred they were able to abolish it by injecting a solution of potassium chloride into the coronary circulation. In later experiments Swan (1954) found that fibrillation could be prevented by intravenous or intracoronary injection of prostigmin (1 ml of a 1:40,000 solution), or by continuous intracoronary injection of acetylcholine. These workers, and many others, have emphasized the danger of air entering the coronary arteries during operations on the open heart, and stress this as one cause of fibrillation; Cookson and Costas-Durieux (1954) have shown experimentally that the danger is greatly reduced by giving an intra-arterial transfusion during the operation at sufficient pressure to keep the aortic valve closed, and refraining from clamping the aorta. Of the other factors concerned little is known. Churchill-Davidson and his colleagues, after extensive investigations (Lynn, Melrose, Churchill-Davidson and McMillan, 1954; McMillan, Melrose, Churchill-Davidson and Lynn, 1955) concluded that during hypothermia the ratio of calcium to potassium in the serum rises, and tentatively suggested that this change, combined with myocardial anoxia due to inadequate coronary flow, might suffice to precipitate ventricular fibrillation or cardiac arrest. As they pointed out, however, further work is required before a definite conclusion can be drawn.

When deep hypothermia is employed ventricular fibrillation constitutes a much greater danger and, in addition, many organs may be severely damaged (Knocker, 1955). Juvenelle and his colleagues (Juvenelle *et al.*, 1952; Juvenelle, 1954) succeeded in cooling dogs to 16°C.,* keeping them at this temperature for 2-3 hours or longer, and subsequently rewarming them, by the ingenious expedient of connecting the animals to a mechanical pump-oxygenator when fibrillation developed and later stopping the fibrillation by means of an electric defibrillator during the rewarming period. As we have seen in Chapter 9 animals of certain species may recover after much more extreme cooling, in some cases to temperatures well below freezing. At present, however, the main interest of this work as far as the surgery of replacement is concerned is in

*One dog recovered after being kept for many hours at 12°C.

relation to the storage of whole organs for grafting (p. 171).

While this experimental work in animals was in progress there were also many important clinical investigations.

Laufman (1951) reported a remarkable case of a patient whose temperature fell to 18° C. as the result of prolonged accidental exposure to sub-freezing temperatures following a bout of heavy drinking, and who subsequently recovered though she sustained severe frostbite necessitating amputation of both legs, one hand and several fingers.

Laborit's technique has been used clinically before, during and after a wide variety of operations (*see* e.g. Bobbio, Goffrini and Bezzi, 1952; Dundee, Gray, Mesham and Scott, 1953) on structures other than the heart and great vessels. The propriety of using hypothermia in such cases has been challenged, but it would take us too far afield to discuss the matter here. It is of interest to note however that Dundee *et al.* observed, apparently contrary to their expectations, that excessive bleeding occurred in some cases.

Cookson, Neptune and Bailey (1952b) reported six patients subjected to a variety of cardiac operations under hypothermia. Two of the patients made a satisfactory recovery, but in neither of them had the circulation been arrested. The first instances of survival of patients after arrest of the circulation and operation on the open heart under hypothermia* were reported in the following year by Lewis and Taufic (1953) and by Swan, Zeavin, Blount and Virtue (1953). Lewis and Taufic reported a single case of an infant with an interatrial septal defect, Swan *et al.* thirteen cases of infants with either pulmonary stenosis or atrial septal defects in which the circulation was stopped for periods ranging from 2 to 8½ minutes, twelve of whom recovered. Further cases were reported subsequently by Bailey and his colleagues (Bailey, Cookson, Downing and Neptune, 1954; Downing, Cookson, Keown and Bailey, 1954) and by Bigelow, Mustard and Evans (1954) who, following Laborit, induced hypothermia by surface cooling combined with administration of Largactil, Phenergan and a sedative drug. The minimum temperature in these patients ranged from 20° to 31° C.

Hypothermia has also been used for cardiovascular surgery in adults, mainly for poor risk patients undergoing cardiac operations (Bigelow *et al.*, 1954), and for the resection of aneurysms of the thoracic aorta (De Bakey and Cooley, 1954; Hardin, Reismann and Dimond, 1954; Cooley and DeBakey, 1955; Eiseman and Summers, 1955; Julian, Grove, Dye, Sadove, Javid and Rose, 1955; Hardin, 1956b) and of the abdominal aorta proximal to the renal arteries (Rob and Eastcott, 1954b). In the first case mild hypothermia is all that is required, the temperature aimed at usually being about 30° C. In operations on the thoracic and upper abdominal aorta a considerably lower temperature may be necessary if hypothermia is to be relied on to prevent serious ischaemic damage, but in adults it has been found dangerous to reduce the temperature below about 26° C., and fatal ventricular fibrillation has been reported to develop even at higher temperatures, for example at 31.1° C. by Downing *et al.* (1954) and at 29° C. by Hardin *et al.* (1954). For this reason most surgeons prefer a bypass technique when applicable, but electrical defibrillation is now so effective that there may well be a swing back to hypothermia.

*[illegible] (1953) had previously reported [illegible] closure of atrial septal defects [illegible] without hypothermia [illegible]

The Establishment of Blood Vessel Banks

In 1945 Blakemore and Lord (1945a) reported briefly some experiments with vein transplants which had been frozen, and two

years later Hufnagel (1947b) reported that segments of aorta in dogs could be excised and successfully replaced by homotransplants which had been sealed in tubes containing helium at a pressure of 10 cm. of mercury, frozen by immersing the tubes in a mixture of ether and solid carbon dioxide, and stored at −70° C.

Gross and his colleagues obtained much less satisfactory results than Hufnagel with frozen grafts, but showed that dog aorta could be successfully grafted, and could also be shown to be viable by a tissue culture test, after storage in a mixture of Hank's solution (p. 13) and 10 per cent homologous serum at 1° to 4° C. for six or seven weeks (Gross, Hurwitt, Bill and Peirce, 1948; Peirce, Gross, Bill and Merrill, 1949; Gross, Bill and Peirce, 1949). They stored human aortic grafts in the same way for periods up to 32 days and used them successfully in the treatment of coarctation of the aorta (Gross, Hurwitt, Bill and Peirce, 1948; Gross, Bill and Peirce, 1949; Gross, 1950, 1951), and may thus fairly claim priority in the establishment of a human blood vessel bank.

Storage by Gross's method has the disadvantage that transplants not used within a few weeks have to be discarded.* The behaviour of frozen homotransplants was therefore further investigated and, despite the unfavourable report of Peirce *et al.* (1949), Hufnagel's observations were confirmed and extended by Deterling and his colleagues (Deterling, Coleman and Parshley, 1950, 1951; Deterling, Parshley and Blunt, 1953), and by Hufnagel himself and Eastcott (Eastcott and Hufnagel, 1951; Hufnagel and Eastcott, 1951, 1952). Deterling *et al.* showed that homografts of dog aorta which had been stored at −70°C. for periods ranging from one day to six months gave excellent functional results irrespective of whether they were thawed quickly or slowly, and whether or not the recipient was given anticoagulants. Grafts stored at −27°C. were less satisfactory but gave good functional results in 12 of 13 animals which received anticoagulants and in 16 of 22 animals which did not. Histological studies showed that degenerative changes occurred rapidly in grafts stored at −27°C., but very slowly in those stored at −70°C.; even the latter, however, were found to be non-viable when tested by explanting sample fragments in tissue culture. Hufnagel and Eastcott set out to compare the effects of rapid and slow freezing, and concluded that rapid freezing gave much the better results; it seems likely, however, that the difference was due to the fact that the rapidly frozen grafts were stored at −70°C. and thawed rapidly, whereas the slowly frozen grafts were stored at −15° to −18°C. and thawed slowly.

In consequence of these encouraging experimental results human frozen artery banks were established, first in London at St. Mary's Hospital by Rob and Eastcott (see Eastcott, 1953; Rob and Eastcott, 1954a), and soon afterwards in many centres in the United States, Britain and other countries.

Arterial segments stored at −70°C. or lower remain functionally effective, though not necessarily viable, for a long time, but transportation is difficult because they must be kept at −70°C. until just before they are to be used. An alternative procedure, in which this difficulty does not arise, is to freeze-dry the transplants (p. 176), for they may then be kept for months, or possibly for years, at room temperature. Freeze-dried artery transplants were first used by Marrangoni and Cecchini (1951), and have since been used extensively both in experimental animals and in man (Hyatt, Turner, Bassett,

* As mentioned in Chapter 9 it is dangerous to use transplants stored by this method for more than six weeks. Hamblin and Lord (1954), for example, reported a case in which death resulted from rupture of an aortic transplant which had been stored for 76 days by this method, whereas a transplant from the same donor to another patient after storage for 42 days was completely satisfactory.

Pate and Sawyer 1952 Pate Sawyer Deterling Blunt and Parshley 1953 Deterling Parshley and Blunt 1953 Hufnagel Rabil and Reed 1953 Pate and Sawyer 1953 Eastcott Holt Peacock and Rob 1954 Creech Cooley and DeBakey 1954 Pate 1954 Rob 1954c Lehr Blakemore Sawyer Glauser and Johnson 1955 Sauvage Wesolowski and Pine 1955) They have proved very satisfactory but there is now some evidence (Rob personal communication) that the long term results are not quite as good as with frozen grafts Apart from this the main disadvantage of freeze dried grafts is that it takes at least half an hour to reconstitute them This usually increases the operating time because most surgeons are reluctant to start reconstituting a graft until it is quite certain that it will be needed owing to the fact that human grafts are in short supply and it seems undesirable to attempt to redry a graft which has been reconstituted

Various methods have been used for sterilizing grafts obtained without aseptic precautions

Meeker and Gross (1951a b) succeeded in sterilizing segments of dog and human arteries without impairing their value as grafts by irradiating them while frozen to −55 C with an electron beam and similar findings were subsequently reported by Hurkeefer Deterling Parshley Humphreys and Glenn (1952) Brunnen (1953) on the other hand had little success with dog aortic grafts sterilized by longer exposure to a less powerful electron beam but this may have been due partly to the fact that his grafts were stored after irradiation at −20 C Present indications are that irradiation can be a satisfactory method of sterilization but that quite small differences in technique may make the difference between success and failure

Hufnagel and his colleagues (Hufnagel Rabil and Reed 1953 Hufnagel 1954) used organic gases with antiseptic properties in particular ethylene dioxide and reported that the method was reliable and did not impair the function of the grafts Ethylene oxide has since been used successfully in many hospitals but is somewhat difficult to handle because it is poisonous and explosive and boils at 10 C Szilagyi and his colleagues (Szilagyi Overhulse Shonnard and LoGuppo 1955 LoGuppo Overhulse Szilagyi and Hartman 1955) therefore tested another substance beta propiolactone which although somewhat toxic is easier and safer to use than ethylene oxide and found it to be very satisfactory

Brunnen (1953) sterilized three dog aortic transplants by immersing them temporarily (for 18 hours) in formalin then transferred them to physiological saline for 24 hours and finally froze them and stored them frozen Two of the three gave excellent functional results when transplanted homologously This work was extended by Ross (1954a) who has reported favourably on the behaviour in dogs of aortic homotransplants which had been sterilized by Brunnen s method and then either frozen or freeze-dried but the procedure does not appear to have stood the test of time and has now been abandoned

Further observations have been made on the behaviour of grafts actually stored in antiseptic solutions including formalin (Peirce Rheinlander Moritz Gross and Merrill 1949 Leger 1953) alcohol (Kimoto 1954) and merthiolate (Mortensen Weed and Grindlay 1954) Some moderately good results were obtained in clinical trials but these procedures are now quite obsolete

Creech DeBakey Self and Halpert (1954) found in dogs that grafts which had been treated with penicillin and streptomycin and then freeze dried were satisfactory as far as infection was concerned * Admittedly as Mortensen *et al* (1954) have

*The long term results were poor owing to the fact that the grafts were heterologous.

pointed out, bacteria of certain genera (notably Pseudomonas and Proteus), and many viruses and fungi are unlikely to be destroyed by this procedure, but it seems to be effective in practice if reasonable care is taken to avoid gross contamination and has been used by Rob (personal communication) and other surgeons in preparing human grafts for clinical use

Blood Vessel Substitutes

The difficulty of obtaining human artery grafts in sufficient quantity has stimulated an intensive search for satisfactory substitutes in the form of either tubes of connective tissue with or without an endothelial lining, or prostheses made of inert material Both rigid and flexible prostheses have been studied, but the latter have proved much the more useful

Tissue Tubes

Sako and his colleagues (Sako, Clatworthy, Chisholm and Varco, 1951; Sako, 1951, Sako and Varco, 1953) implanted pericardial autografts into the thoracic aortas of adult dogs The transplants tended to dilate if they were unsupported but could be prevented from doing so by wrapping them in fascia lata Zech, Nyhus, Griffith and Harkins (1955) subsequently used pericardial autografts to replace segments of the thoracic aorta in pigs Some of the grafts were unsupported while others were wrapped in fascial tissue obtained from the central tendinous portion of the diaphragm. All the grafts remained patent but many, including some of the wrapped grafts, showed marked aneurysmal dilatation, and it was concluded that pericardium with or without a fascial wrapping was not a very satisfactory arterial substitute.

Other investigators have used tubes prepared by burying a piece of polyethylene tubing in the subcutaneous tissue (Fontaine, Kim and Kieny, 1953) or within the rectus sheath (Peirce, 1953). In the course of a few weeks the polyethylene becomes surrounded by tough connective tissue with a smooth lining closely resembling endothelium in structure. The tissue tube is then dissected out and the polyethylene splint is discarded. It has been shown that such tubes will function, at any rate for a time, as arterial substitutes, but the procedure is complicated and time-consuming, and is of historical interest only.

Rigid Prostheses

The first successful attempt to restore continuity of the aorta permanently with a rigid prosthesis was reported by Hufnagel (1947a). Prior to this, as we have seen, Carrel (1912b, c) had used tubes of glass, aluminium, and gold plate to bridge gaps in the thoracic aorta in animals, and Tuffier's tubes were used during the first world war to bridge gaps in peripheral arteries in men wounded in battle, but thrombosis occurred sooner or later in every case.

According to Hufnagel (Hufnagel and Gillespie, 1951a; Hufnagel, 1955), if a rigid prosthesis is to function permanently when used to bridge a gap in the aorta or a large artery the material must be to a great extent biologically inert, must be sufficiently strong to withstand the stresses to which it is subjected in the body, must remain sufficiently strong throughout the life of the recipient, and should be water-repellant. Among the many materials tried methyl methacrylate (Lucite, Plexiglas) proved to be the most satisfactory.

In addition, the anastomoses between vessel and tube must be smooth, and must be made in such a way that necrosis of the arterial wall does not occur at the junction. To achieve this Hufnagel and his colleagues (Hufnagel, Harvey, Rabil and McDermott, 1954) introduced the principle of "multiple point fixation" (Fig. 146), which they have now used in a total of over 400 animals and patients.

Hufnagel has claimed that replacement

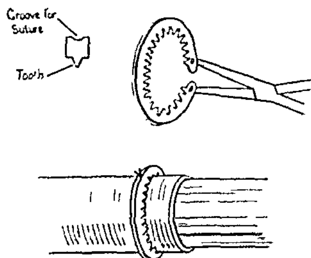


Fig. 116 Hufnagel's method of joining the aorta to a rigid prosthesis by multiple point fixation. The toothed plastic ring is slipped over the end of the aorta and tightened by an encircling suture after the prosthesis has been inserted

by a rigid prosthesis with multiple point fixation is extremely satisfactory in appropriate sites. It is unsatisfactory with very small arteries and, of course, where the gap to be bridged crosses a major flexion crease. A disadvantage of the method is that it is necessary to have a very large selection of tubes varying both in diameter and length and rigid prostheses have now been abandoned in favour of flexible ones except in the special case where a rigid prosthesis incorporating a valve mechanism is used in Hufnagel's operation for the treatment of aortic incompetence (Hufnagel *et al.*, 1954).*

Flexible Prostheses

The feasibility of bridging arterial defects with tubes of flexible woven material was first demonstrated by Voorhees, Jaretzki and Blakemore (1952) who used tubes of a material known as Vinyon N cloth. Following this report many materials have been tested including polyvinyl sponge (ivalon), silicone rubber, nylon, orlon, terylene (Dacron®)† and teflon.

Initially surgeons made their own synthetic fibre prostheses by rolling a piece of the chosen material into a tube of appropriate size and sewing the seam by hand or with an ordinary sewing machine, but it was not very long before manufactured prostheses of various materials, some with seams and some seamless, became available commercially (*see* Hufnagel, 1955).

It became apparent that the effectiveness of a prosthesis depends not only on the substance from which it is made but also on the type of weave and other physical characteristics of the material, and a vast amount of work has been done investigating this matter. An important development was the introduction of seamless chemically treated crimped nylon prostheses (Fig. 117) by Edwards and Tapp (1955, 1956), and this has been followed by the development of crimped prostheses of dacron and teflon.

In 1957 there was considerable difference of opinion as to the relative merits of the various prostheses then available but, despite some opinion to the contrary (*see* e.g. Edwards, 1957a, b), most surgeons believed that while some of them were satisfactory for aortic replacement they were all very much inferior to homografts for replacing or bypassing vessels of the calibre of the femoral artery (*see* e.g. DeBakey and Crawford, 1957; Rob, 1957a). Today the situation is different and the short term results available suggest that crimped teflon prostheses will prove effective in all operations in which homografts are now normally used. It remains to be seen whether the long term results will be equally satisfactory and also whether prostheses will give results comparable to homografts if the scope of vascular replacement is extended to include vessels such as the anterior and posterior tibial arteries and the coronary arteries.

Prostheses have become so important as

* This operation also is becoming obsolete except for the fact that the original rather noisy valve has been replaced by one which is less audible to the patient.

† This material was developed in Britain and named terylene. In the United States, where it is manufactured under licence, it is called *Dacron*®.

substitutes for arterial homografts that some discussion of them has been essential, but they do not really fall within the scope of this book, and for a full account of their development the reader is referred to Edwards' (1957b) monograph.

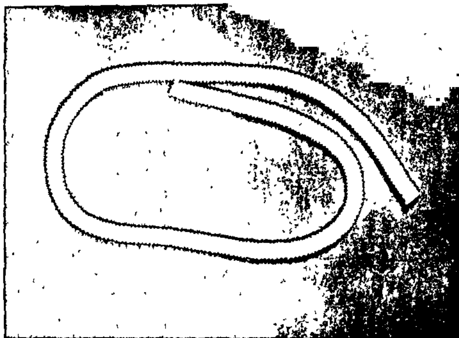


Fig. 117 Edwards Tapp crimped nylon artery prosthesis.

THE FATE OF BLOOD VESSEL GRAFTS

As we have seen (p. 12) vessel homografts, even if viable, do not survive but are in part replaced by host tissue and in part persist as inert material. The process of replacement, or re-organization as it may be termed, has been discussed in Chapter 3 Fresh autografts may survive in part as living tissue but they too appear to be replaced to a considerable extent by tissue of local origin.

When vessel grafts are used in young patients, as in the treatment of some cases of coarctation of the aorta, it is important that *the re-organized graft should grow pari passu* with the host vessel. Experiments in growing animals (dogs and pigs) suggest that grafts which remain patent are capable of growing both in length and diameter (Everson and Southwick, 1951), but do not as a rule keep pace with the host vessel (Johnson, Kirby and Horn, 1952, Kanar, Nylus, Schmitz, Sauvage, Moore, Zech and Harkins, 1951), and according to Kanar *et al.* the discrepancy is greater with grafts in the thoracic aorta than with those in the abdominal aorta. Clinically however the demand for growth is rarely if ever of the same magnitude as in these experiments, and the problem is not as serious as it might appear (*see* p. 136).

The incidence of degenerative changes in grafts is also important. Calcification and atheroma-like degeneration have been observed in *experimental grafts in dogs* and pigs by many observers (Coleman, Deterling and Parshley, 1951; Everson and Southwick, 1951; Johnson, Kirby and Horn, 1952; Parsons, Gerbode and Cox, 1952; Gentsch, Waters and Glenn, 1951; Nylus, Kanar, Moore, Schmitz, Zech, Sauvage and Harkins,

1955 and others) and it did not appear to make very much difference whether the grafts were fresh, frozen or freeze-dried. According to Sylius *et al.* experimental grafts in the thoracic aorta are more prone to degeneration than those in the abdominal aorta, but clinically homografts in this situation have been remarkably satisfactory although, as one would expect, degenerative changes certainly do occur.

BLOOD VESSEL GRAFTS AND PROSTHESES IN PRESENT DAY SURGERY

CHOICE OF MATERIAL

Fresh vein autografts are useful in an emergency but for routine use the choice lies between *arterial homografts* and *prostheses*. The respective merits of these have already been discussed (p. 433).

Homografts are usually either *stored* frozen at −79° C. or freeze-dried and, as we have seen, may be taken aseptically, taken *with care to avoid gross contamination* and treated with antibiotics, or taken without special precautions and sterilized by *exposure to ethylene oxide, beta propiolactone* or irradiation.

Prostheses are of many kinds. Crimped *dacron prostheses are very satisfactory in the aorta* but even in this situation crimped teflon prostheses are at least as good and elsewhere appear to be very decidedly better.

INDICATIONS FOR USE

The indications* for using blood vessel grafts and prostheses will be considered under the following headings:

1. Coarctation of the aorta
2. Recent arterial injuries
3. Aneurysms of the aorta and major arteries
4. Traumatic arteriovenous fistula
5. Segmental obstruction due to occlusive arterial disease
   (1) Thrombosis at the aortic bifurcation
   (2) Stenosis of the renal artery associated with hypertension
   (3) Femoral and popliteal occlusion
   (4) Occlusion of the internal carotid artery
   (5) Coronary occlusion
6. Primary arterial thrombosis
7. Neoplasms affecting main arteries
8. Other conditions

*Useful general reviews of the indications for blood vessel transplantation have been published by DeCamp (1953), Keeley and Moore (1953) and Brown, Hufnagel, Pate and Strong ([illegible]).

Coarctation of the Aorta

In a proportion of patients with coarctation of the aorta there is an associated aneurysm or the constricted segment is so long that excision and end-to-end anastomosis is impossible. Under these circumstances the best procedure is to excise the affected segment and replace it by a graft or prosthesis. The operation is normally performed without employing a temporary shunt or using hypothermia, and provided only the aorta is clamped there appears to be no danger of paraplegia. There have, however, been two cases reported in which paraplegia developed following clamping of both the aorta and the left subclavian artery (Bing, Handlesman, Campbell, Griswold and Blalock, 1948; Beattie, Nolan and Howe, 1953).

Excellent immediate results have been obtained first by Gross (Gross, Hurwitt, Bill and Peirce, 1948; Gross, Bill and Peirce, 1949; Gross, 1950, 1951) and subsequently by Hufnagel and Gillespie (1951b), Brock (1953), Deterling (1953) and other surgeons. So far, moreover, the long

term results appear to be good but, as Gross (1951) has pointed out, "no final conclusions should be made until these patients have been followed for several decades." The risk of progressive relative narrowing at the site of anastomosis in a growing vessel does not appear to be great, especially if part at least of the anastomosis is made with interrupted sutures (*see* Hurwitt and Brahms, 1951, Johnson, Kirby, Allam and Hagan, 1951a, Hurwitt and Altman, 1954), there is however some doubt about the ability of the transplant as a whole to keep pace with the growth of the host vessel.

Recent Arterial Injuries

When an artery is cleanly divided, and the patient, after bleeding has been temporarily controlled, is admitted promptly to hospital, it is sometimes possible to restore continuity by immediate end-to-end anastomosis In other cases, where the vessel is only partly divided, it may sometimes be repaired by lateral suture Usually, however, when there is a wound in a main artery the choice of treatment lies between ligation on the one hand, and, on the other hand, excision of the damaged segment of vessel and replacement by a graft or prosthesis

The danger of ligation, especially of the common femoral artery and the popliteal artery, became apparent during the first world war (Makins, 1919), but until recently there was no satisfactory alternative (*see* e. g DeBakey and Simeone, 1946). During the Korean campaign, however, autologous vein transplants were used successfully in favourable cases to replace damaged portions of main arteries (Cooke, Hughes, Jahnke and Seeley, 1953; Seeley, Hughes and Jahnke, 1953), and nowadays some form of replacement should be used in preference to ligation of the common femoral or popliteal artery when simple suture is impossible and the state of the wound permits (*see* e.g. Jahnke and Howard, 1953; Jordan and Wilson, 1953).

Aneurysms of the Aorta and Major Arteries

The surgical methods which have been used for the treatment of aneurysms may be classified as follows:*

1 Arterial ligation.
2. Introduction of wire to induce clotting.
3. Wrapping in cellophane and other procedures designed to evoke a fibroblastic reaction around the sac.
4. Excision of the sac and ligation of the artery above and below.
5. Endo-aneurysmorrhaphy.
   (1) Obliterative.
   (2) Restorative.
   (3) Reconstructive.
6. Excision and reconstruction.

Arterial ligation is now virtually obsolete because, as Rob (1954b) has expressed it, wherever the ligature was placed, "if the method was effective in producing a firm clot in the aneurysm then the risk of gangrene was considerable, and if the limb survived the aneurysm remained because of the efficient collateral circulation."

Introduction of wire (*see* Linton, 1951, and Cooley and DeBakey, 1952, for review), and *wrapping with cellophane or polyethylene* (Poppe, 1949) have been used until comparatively recently in the treatment of aneurysm of the aorta but cannot be said to provide a radical cure (*see* discussion in Cooley and DeBakey, 1952).

Excision of the sac and ligation of the artery above and below cures the condition

*The classification used here is that given by Cooley and DeBakey (1952) and Rob (1954b) Of course, in addition to surgical treatment, medical treatment may be required to deal with the underlying cause of the condition.

A useful review of the treatment of aneurysm of the thoracic aorta and its main branches prior to the introduction of excision and replacement has been published by Borrie and Griffin (1950).

and is the treatment of choice when the vessel can safely be sacrificed. In practice, except in the case of small and unimportant arteries, this condition is fulfilled only occasionally, notably in relatively young patients with a traumatic aneurysm of a medium sized artery such as occurs from war wounds (*see* Bigger, 1944; Elkin, 1944; Freeman, 1946). Even when there appears to be no danger of gangrene the procedure must be used with discrimination because it may be followed by ischaemic contracture or claudication. It has been used successfully in the treatment of an aneurysm of the right subclavian artery (Cooley and DeBakey, 1952) but it is unlikely that it would be used for this purpose today.

The introduction of *endo-aneurysmorrhaphy* by Matas in 1888 and its subsequent elaboration (*see* Matas, 1903, 1921) was an important development, and as recently as 1952 Cohen expressed the generally held view when he stated that for traumatic aneurysms of larger vessels *obliterative aneurysmorrhaphy* was usually "the operation of choice and necessity," but these procedures are now becoming obsolete. The obliterative operation has the same disadvantage as excision without reconstruction, and offers less certainty of cure; the restorative and reconstructive operations are much safer but are often followed by recurrence of the aneurysm.

Excision and reconstruction is the treatment of choice when excision without reconstruction would be unsafe, provided that the operation is technically possible and the general condition of the patient is satisfactory.

The reconstruction may take either of two forms.

With some saccular aneurysms it is possible to excise the aneurysm without resecting a segment of the artery and close the resulting defect by suture. This type of operation has been used successfully in the treatment of saccular aneurysm of the aorta, first by Ochsner (1944) and subsequently by Monod (*see* Meyer, *Monod, Brunel, Nico and Dubois de Montreinaud*, 1948; Monod and Meyer, 1950), Cooley and DeBakey (1952, 1953), and Bahnson (1953a, b).

With other saccular aneurysms and with fusiform* aneurysms, on the other hand, a segment of the vessel has to be resected and the resulting gap must then be bridged by a graft or prosthesis. As we have seen, resection of an arterial aneurysm and replacement by an autologous vein graft was carried out successfully by Lexer and Pringle before the first world war (p. 115) and on many occasions between the wars by Weglowski and other surgeons (p. 116), but the full potentiality of the method was not realized until much more recently, when homografts and prostheses suitable for use in vessels ranging in size from the aorta to the popliteal artery became available. By the end of 1955 however resection and replacement had been used by many surgeons† for the treatment of aneurysms of the thoracic aorta associated with coarctation (Swan, Maaske, Johnson and Grover, 1950; Gross, 1951; Brock and Graham, 1952; Rob, 1954b), aneurysm of the descending thoracic aorta or distal aortic arch not associated with coarctation‡ (Lam and Aram, 1951;

* As Bahnson (1953b) has pointed out, in the aorta syphilitic aneurysms are usually saccular and situated above the renal arteries, whereas arteriosclerotic aneurysms are fusiform and are usually situated below the renal arteries.

†In the bibliography which follows reference is made to a number of papers which appear in retrospect to have been of special importance, but no attempt has been made to cite all the recorded cases.

‡Most of these cases were treated with the aid of either a shunt (Lam and Aram, 1951; Johnson *et al.*, 1955) or hypothermia (DeBakey and Cooley, 1954; Cooley and DeBakey, 1955; Julian *et al.*, 1955). But in the first case reported by DeBakey and Cooley (1953a) neither was used and the patient recovered without residual disability after the aorta had been clamped for 45 minutes. This would seem to be a remarkable instance of beginners' luck.

The patient operated on by Lam and Aram died three

→

DeBakey and Cooley, 1953a, 1954, Cooley and DeBakey, 1955; Johnson, Kirby and Lehr, 1955; Julian, Grove, Dye, Sadove, Javid and Rose, 1955; Stranahan, Alley, Sewell and Kausel, 1955); aneurysm of the abdominal aorta (Dubost, Allary and Oeconomos, 1952, Bahnson, 1953b, 1954, Brock, 1953, DeBakey and Cooley, 1953b, Dubost, Oeconomos, Lemoine and Robert, 1953; Gerbode, Parsons and DaCosta, 1953, Helden, Kirklin and Gifford, 1953, Kirklin, Waugh, Grindlay, Openshaw and Allen, 1953, Dubost and Dubost, 1954 Rob and Eastcott, 1954b; Rob, 1955); and aneurysm of peripheral arteries* (Martin and Lynn, 1952, Haguenau, Oudot, Rogé, Jany, Delahaye and Hirsch, 1954, Rob, 1954b) The case of Haguenau *et al* is of special interest in that hypothermia was used to reduce the risk of hemiplegia in a patient with a carotid aneurysm which was resected and replaced by a transplant joining the common and internal carotid arteries.

A modified procedure, which was used successfully by Mahorner and Spencer (1951a, b) in treating an aneurysm of the innominate artery, is to ligate the vessel proximal and distal to the aneurysm after attaching a graft as a permanent shunt, and to excise the sac later if necessary. A similar technique was used later by Adams (1955) in treating an aneurysm of the distal part of the aortic arch.

An attempt by Cooley, Mahaffey and DeBakey (1955) to resect the entire aortic arch using a temporary bypass was unsuccessful, the patient dying of cerebral ischaemia, but now it is feasible to resect an aneurysm in any part of the aorta (Cooley, DeBakey and Morris, 1957).

For the descending thoracic aorta a temporary shunt from the left auricle to the femoral artery incorporating a pump, as described by Gerbode, Braimbridge, Osborn, Hood and French (1957) and Morris, Witt, Cooley, Moyer and DeBakey (1957), is probably the safest method. The main risk is ischaemic damage to the spinal cord, which may occur whatever the operative technique if a significant part of the blood supply of the cord is from vessels arising from the part of the aorta which is resected.

The same technique or hypothermia may be used in conjunction with resection of the upper part of the abdominal aorta.

Resection of the ascending aorta was first accomplished successfully by Cooley and DeBakey (1956) using cardio-pulmonary bypass. The bypass was begun with total inflow occlusion. Most of the blood was returned to the arterial system via a catheter threaded into the abdominal aorta through the right femoral artery, but in addition some was led into a small catheter placed in the right common carotid artery for cerebral perfusion at 200 c c./min. during occlusion of the aorta and the innominate artery. The aorta was cross clamped at the coronary isthmus. Distally the aortic arch and innominate artery were occluded proximal to the left common carotid. The ascending aorta, which was the site of a fusiform aneurysm, was resected and replaced by a homograft. Ten minutes after the bypass was started the heart stopped and no attempt was made to start it until the aortic clamps were removed after 31 minutes' perfusion. Cardiac massage was then started with the pump oxygenator still functioning. Ventricular fibrillation ensued but sinus rhythm was restored after two shocks from an electric defibrillator and the bypass was dismantled. The patient subsequently made an excellent recovery.

months after operation from suppuration within the sac which had been incompletely removed, the operation was thus not an unqualified success, but it was, as Brock (1953) has remarked, a notable achievement nevertheless.

*As mentioned earlier, excision of aneurysms of peripheral arteries and restoration of continuity by vein autografts was performed successfully before the first world war in Germany and also in Britain

It should be possible by perfusing both carotids to resect the entire aortic arch

Traumatic Arteriovenous Fistula

Traumatic arteriovenous fistulae are as a rule the result of war wounds and occur but rarely in time of peace

Until recently the usual treatment was quadruple ligature (*see* e g Bigger 1944 Cohen 1952) but this has now been given up in favour of excision and arterial replacement—a procedure used as long ago as 1913 by Coenen and revived by Shumacker (1948) Gerbode Holman Dickenson and Spencer (1952) and other surgeons Moreover as Rob and Eastcott (1953) have shown it is possible to relieve claudication resulting from quadruple ligature performed many years previously by inserting an arterial graft

Segmental Obstruction Due to Occlusive Arterial Disease

Many of the serious consequences of generalized arterial disease are due in the first instance to localized occlusion or stenosis involving a comparatively short segment of an important vessel and when this is the case the question of relieving the obstruction surgically naturally arises

The procedures which have been used are as follows (a) thrombo endarterectomy (b) resection and anastomosis (c) resection and replacement by a graft or prosthesis and (d) insertion of a graft or prosthesis to bypass the obstruction

The general history of the development of methods of arterial anastomosis and replacement has already been considered but a word must be said about the development of thrombo-endarterectomy According to Reboul and Laubry (1950) Lejars performed the first disobliterative operation for arterial thrombosis in 1902 but the real possibilities of thrombo-endarterectomy were first demonstrated by Dos Santos (1949) who performed the operation through a series of transverse incisions in the vessel and used heparin locally during the operation and systemically afterwards to reduce the risk of further thrombosis The operation has been modified in various ways Reboul and Laubry (1950) used a longitudinal incision in the vessel Wylie and his colleagues (Wylie Kerr and Davies 1951 Wylie and Gardener 1955) advised wrapping the disobliterated segment with fascia to reduce the risk of dilatation or rupture and Cannon has devised an intraluminal ring stripper which is available in various sizes and makes it possible to deal with a long obstruction often through only two small incisions (Cannon and Barker, 1955 Cannon Barker and Kawakami 1958)

We shall now consider in detail the place of these various operations in the treatment of segmental obstruction due to occlusive arterial disease at a number of different sites in the arterial system

Thrombosis of the Aortic Bifurcation and Distal Abdominal Aorta

Thrombosis of the aortic bifurcation is mentioned in Allbutt and Rolleston s *System of Medicine* (1909) but the first full accounts of the lesion and its clinical features were given by Leriche (Leriche, 1923 1940 Leriche and Morel 1948)

There may at first be only mild symptoms such as undue liability to fatigue and vague pain in the legs later however there is intermittent claudication pallor and coldness of the feet and legs absence of arterial pulsation in the lower extremities muscular atrophy and finally gangrene In males there may in addition be sexual impotence The diagnosis though it may be suggested by the symptoms and clinical findings is usually made by aortography (Fig 118)

Leriche suggested that the ideal treatment would be to resect the affected portion of the aorta and its bifurcation and replace this by a graft He held however,

1938; Lewis and Goldblatt, 1942) that hypertension could be produced experimentally in animals by constricting one or both renal arteries. In consequence of this discovery surgeons gradually ceased ligating aberrant renal arteries for the treatment of hydronephrosis, and succeeded in relieving hypertension in a small proportion of patients with unilateral renal disease by nephrectomy.

The development of aortography opened the way to treatment of a more conservative kind, and Freeman, Leeds, Elliott and Roland (1954) succeeded in relieving hypertension in a patient with partial occlusion of the aorta and left renal artery by thrombo-endarterectomy. Subsequently conservative operations on the renal artery, including resection and anastomosis, replacement by a graft, and thrombo-endarterectomy have been used with remarkable results in a large series of patients with hypertension associated with renal ischaemia due to arterial stenosis in whom nephrectomy appeared to be contra-indicated either because the renal function was so good or because the condition was bilateral. The leaders in this field have been Poutasse and his colleagues at the Cleveland Clinic (Poutasse, 1956; Poutasse, Humphries, McCormack and Corcoran, 1956; Humphries and Poutasse, 1957; Poutasse and Dustan, 1957; Poutasse, Engel and Dustan, 1957). The technique which Poutasse *et al.* have found most satisfactory is to divide the aorta below the renal arteries, insert a homograft consisting of a segment of

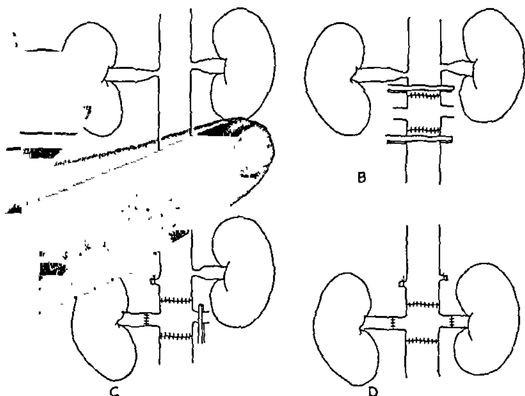


Fig. 149. Method of restoring an adequate arterial supply to both kidneys in a patient with atherosclerosis involving both renal arteries. A. Diagram showing narrowing of both renal arteries close to their origins B The aorta has been divided and a homograft consisting of a segment of aorta with a small piece of each renal artery has been inserted Note that the original blood supply to the kidneys has been maintained during this stage of the operation. C. The host's right renal artery has been divided and the kidney has been connected to the renal artery of the graft. D. The left kidney has been dealt with in the same way as the right one (After Humphreys and Poutasse.)



Fig. 118. Translumbar aortogram showing occlusion of the abdominal ... below the origin of the renal arteries. The patient complained of ... claudication and no pulses could be felt in the abdomen or the lower extremities.

that ... ation was not technically possible ... therefore practised instead bilateral ... bar sympathectomy combined in good ... patients with resection of the affected vessels but without any attempt at reconstruction. This form of treatment or lumbar sympathectomy alone was used also with some success by Ortner and Griswold (1950) while others (West, Schetlin and Schilling 1953) performed thrombo-endarterectomy, also with success.

The feasibility of replacing the aortic bifurcation by a graft was first demonstrated by Oudot (Oudot 1951a, b; Oudot and Beaconsfield 1953) and following this further successful cases were reported by Brock (1953), Julian, Grove, Dye, Olwin and Sadove (1953), Giberson, Waugh, Hines and Faulconer (1954) and DeBakey, Creech and Cooley (1954). Since then the operation using either a graft or a prosthesis has been performed many times and is the standard form of treatment in patients whose general condition is satisfactory.

Stenosis of the Renal Artery Associated with Hypertension

It was shown many years ago by Goldblatt and his colleagues (Goldblatt, Lynch, Hanzal and Summerville, 1934; Goldblatt

1938; Lewis and Goldblatt, 1942) that hypertension could be produced experimentally in animals by constricting one or both renal arteries In consequence of this discovery surgeons gradually ceased ligating aberrant renal arteries for the treatment of hydronephrosis, and succeeded in relieving hypertension in a small proportion of patients with unilateral renal disease by nephrectomy.

The development of aortography opened the way to treatment of a more conservative kind, and Freeman, Leeds, Elliott and Roland (1954) succeeded in relieving hypertension in a patient with partial occlusion of the aorta and left renal artery by thrombo-endarterectomy. Subsequently conservative operations on the renal artery, including resection and anastomosis, replacement by a graft, and thrombo-endarterectomy have been used with remarkable results in a large series of patients with hypertension associated with renal ischaemia due to arterial stenosis in whom nephrectomy appeared to be contra-indicated either because the renal function was so good or because the condition was bilateral. The leaders in this field have been Poutasse and his colleagues at the Cleveland Clinic (Poutasse, 1956; Poutasse, Humphries, McCormack and Corcoran, 1956; Humphries and Poutasse, 1957; Poutasse and Dustan, 1957; Poutasse, Engel and Dustan, 1957). The technique which Poutasse *et al.* have found most satisfactory is to divide the aorta below the renal arteries, insert a homograft consisting of a segment of

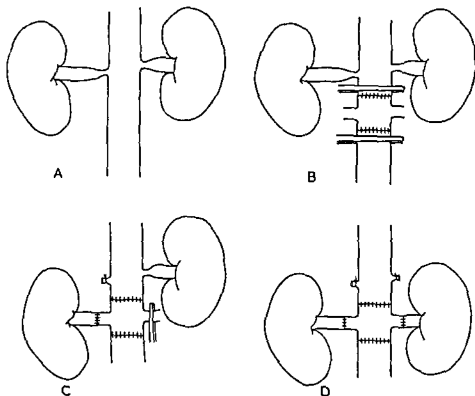


Fig. 149. Method of restoring an adequate arterial supply to both kidneys in a patient with atherosclerosis involving both renal arteries A. Diagram showing narrowing of both renal arteries close to their origins. B. The aorta has been divided and a homograft consisting of a segment of aorta with a small piece of each renal artery has been inserted. Note that the original blood supply to the kidneys has been maintained during this stage of the operation. C. The host's right renal artery has been divided and the kidney has been connected to the renal artery of the graft. D. The left kidney has been dealt with in the same way as the right one (After Humphreys and Poutasse.)

Moran, 1952b) or other tissues to the myocardium, or (c) implanting the divided internal mammary artery in the myocardium (Vineberg 1946, 1951, Vineberg and Jewett, 1947, Glenn and Beal, 1950)

2 Attempts to increase the flow of arterial blood to the myocardium by connecting the coronary sinus to a systemic artery or, via a graft, to the aorta (Beck, Stanton, Batiuchok and Leiter, 1948, Beck 1948), or by simple ligation of the coronary sinus

3 Operations designed to remove the obstruction including thrombo endarterectomy (Longmire, Cannon and Kattus, 1958), and resection and grafting

Operations designed to remove the obstruction would seem to offer the best if not the only, chance of success and Longmire, Cannon and Kattus (1958) have had encouraging results with thrombo endarterectomy in a few patients with crippling anginal pain, but as would be expected the mortality has been high It seems likely that coronary arteriography would be helpful in selecting cases for surgical treatment and planning the operation, and that the hazards of the procedure could be reduced by using hypothermia or cardio pulmonary bypass but it remains to be seen whether the mortality can be reduced, and the prospects of lasting benefit increased to the point where the operation becomes generally acceptable

Primary Arterial Thrombosis

Thrombosis of the femoral popliteal or brachial artery occurs occasionally in patients in their late twenties or thirties in whom there does not appear to be any generalized arterial disease (Learmonth, Blackwood, and Richards, 1944) Severe claudication may result, and in such cases excision of the affected segment of artery and replacement by a graft or a bypass operation is indicated Eastcott in 1953 reported one patient with primary thrombosis of the right popliteal artery in whom resection and replacement was brilliantly successful, another with thrombosis of the right common and external iliac arteries who was considerably improved, and a third with thrombosis of the left external iliac and common femoral arteries who was slightly improved after operation

Neoplasms Affecting Main Arteries

Cade (1951) has made a strong case for treating the majority of soft tissue sarcomata of the limbs, especially fibrosarcomata, by a combination of deep X ray therapy and wide local excision It sometimes happens that the tumour, especially if it is situated in the thigh, is adherent to the main artery, and in such cases adequate local excision may still be possible if the involved segment of artery is excised with the tumour and replaced by a graft or prosthesis Three cases in which this was done have been reported by Swan and Morfit (1951) The same problem may arise with tumours in the neck involving the bifurcation of the common carotid artery (Conley, 1953) Sometimes also it is desirable to resect a segment of artery when performing a block dissection of lymph nodes, especially in the groin, and again replacement may be indicated

Other Conditions

Arterial grafts have been used by Gross (see Gross, Hurwitt, Bill and Peirce, 1948 Gross, Bill and Peirce, 1949) as a bridge between the aorta or one of its main branches and the pulmonary artery for the relief of the pulmonary stenosis in patients with *Fallot's tetralogy*, when it appeared impossible to perform a direct anastomosis of either the Blalock or Potts type, but as we have seen all these procedures are now being replaced by a more direct type of attack

A rigid prosthesis incorporating a valve mechanism is inserted in the aorta just distal to the left subclavian artery in Huf

nagel's operation for *aortic incompetence* (p. 433), but this operation too appears to be becoming obsolete.

Finally, venous or arterial grafts may have a use in the treatment of *obstruction of the vena cava,* and occasionally also in creating a porto-caval shunt in patients with *portal hypertension.*

TECHNIQUE

The technique of blood vessel transplantation varies according to the condition for which the operation is being performed and the site in which the transplant is to be placed. We shall therefore consider the general principles to be followed and some examples of their application.

General Principles

General anaesthesia is the rule, and since transplantation operations are usually prolonged it is especially important to keep the patient well oxygenated and to use the minimum quantity of anaesthetic consistent with the needs of the surgeon. If the chest is to be opened it is, of course, necessary to use a cuffed intratracheal tube so that respiration can be controlled, and the anaesthetist must be constantly on the alert in case the opposite pleural cavity is opened without warning. An intravenous infusion should be set going before the operation is started and an adequate quantity of blood—at least six pints for operations on the aorta—should be ready *in the operating theatre* in case of sudden haemorrhage.

For replacement of the ascending aorta or the aortic arch some form of cardiopulmonary bypass using a pump-oxygenator is required.

For operations on the unobstructed descending aorta or the abdominal aorta above the level of the renal arteries,* the choice lies between a pump-assisted bypass from the left auricle to the distal aorta or the femoral artery (*see* Fig. 151) and hypothermia, and present indications are that the shunt gives better protection than cooling to 28° or 29°C. (Rob, 1958). A simple shunt from upper to lower aorta is theoretically possible and has been tried, but is less reliable and more difficult to manage.

A similar choice applies for operations on the internal and common carotid arteries, but here hypothermia is probably the simpler method and appears to be quite adequate.

The various methods which have been used for inducing hypothermia have been discussed already (p. 426), but it remains to emphasize a number of important practical points. External cooling is the simplest method, and it is most convenient to use a special blanket, such as the ones described by Bigelow, Lindsay, Harrison, Gordon and Greenwood (1950) and Inglis, Biffen and D'Abreu (1954), provided with continuous rubber or plastic tubing through which fluid† can be circulated at any desired temperature; it is, however, quite safe to immerse the patient in a bath at a temperature of 4°-6°C., or to cool him in air in a refrigerated chamber. As an alternative to external cooling the veno-venous shunt of Brock and Ross (p. 428) may be used. Before cooling the patient is, of course, anaesthetized, and a cuffed tube is inserted into the trachea. Prevention of shivering during cooling is most important, and short-acting relaxants such as succinylcholine are useful in this connexion. The temperature is measured by a thermocouple or a thermistor in the rectum, and plotted on a graph every five minutes; as a rule the

*When there has been chronic obstruction as in coarctation the thoracic aorta can be clamped, according to Cooley and DeBakey (1955) "for almost indefinite periods of time" without hypothermia; as we have seen (p. 435), however, paraplegia has occurred after clamping both the aorta and the left subclavian artery even in [illegible] of coarctation. The pre[illegible] [illegible]minal aorta can safely be [illegible] [illegible]ies for at least three hours

† [illegible] water and ethylene [illegible]

temperature is not allowed to fall below 26°C An electrocardiograph is connected to the patient throughout the operation and if cardiac irregularities are observed the patient s temperature should be raised lest ventricular fibrillation develops Intravenous fluids should be administered sparingly especially according to Wynn (1954) those containing glucose During the operation haemostasis must be scrupulous because the clotting time is prolonged under hypothermia and troublesome bleeding may occur from quite small vessels At a temperature of 28°C it appears to be safe to clamp the aorta just distal to the left common carotid artery for an hour (Cooley and DeBakey 1955) Under the same conditions Rob and Eastcott (1954b) have clamped both renal arteries and the abdominal aorta for 59 minutes and the aorta at the level of the diaphragm the coeliac axis the superior mesenteric artery both renal arteries and a number of lumbar and lower intercostal arteries for 1 hour 57 minutes with no permanent ill effect in either case

If a temporary shunt is used the patient is heparinized but the clotting time may be restored to normal by an injection of protamine after the shunt has been dismantled

Adequate mobilization of the host vessel is essential and this is aided by generous exposure and by the judicious use of tapes or better still Penrose tubing passed round the vessel The accompanying vein must be carefully preserved and care should be taken not to interfere more than is absolutely necessary with the collateral circulation

When the host vessel has been mobilized it is clamped above and below and in addition any collaterals entering the isolated segment are also clamped The various clamps available have already been considered The author prefers tape tourniquets to clamps for controlling arteries of the calibre of the femoral and Crafoord s clamp for the aorta but Potts ductus and aortic clamps and a clamp with similar teeth known as Southwick s clamp (*see* Julian Grove and Norberg 1954) are very reliable and should be available for use in an emergency Heparin is injected into the vessel distal to the isolated segment or alternatively heparin is given intravenously and when a segment has been resected the ends of the vessel above and below are flushed out with heparin saline The adventitia is stripped from the vessel in the region of the anastomosis

If a graft is being used its length should be such that when it is in place it will be under a moderate degree of tension this is because a graft stretches when the blood flow is restored and if it has not been sutured under tension redundant curves form in which clotting can occur (Rob 1954b) Vein transplants must be orientated so that the valves permit blood to flow in the proper direction with arterial transplants however orientation does not matter except when the transplant tapers The adventitia is stripped back for about half an inch from both ends of the graft before the anastomoses are started

Prostheses of the crimped pattern now in common use are also inserted under tension Some types—depending on the weave and the material used—must be steeped in fresh blood to render the fabric less permeable and so reduce blood loss when the clamps are removed With some materials the ends must be sealed with a cautery to prevent fraying

For most purposes a simple continuous over and over stitch is used but in children interrupted sutures are often used In the aorta a two layer anastomosis is sometimes advisable Most surgeons use 4/0 silk for the aorta and 5/0 or 6/0 silk for peripheral arteries The material is lightly coated with liquid paraffin so that it runs nicely Atraumatic needles are commonly used but ordinary intestinal needles being tougher are

useful for suturing an atheromatous aorta.

When both anastomoses are complete the distal clamp is released first and then the proximal. If, as often happens, a little blood leaks out, the suture line is gently compressed between finger and thumb for a few minutes. This usually suffices to stop the bleeding, but if it does not the clamps are re-applied and one or two additional sutures are inserted.

It is sound practice where possible to wrap a prosthesis temporarily in polyvinyl alcohol sponge or other haemostatic material in order to limit blood loss when the clamps are first released. Some surgeons wrap their grafts and anastomoses in gelatin sponge and leave this material in place on the ground that it stimulates the formation of firm fibrous tissue (Hurwitt, 1953), but others feel that this is harmful because it delays revascularization.

There is considerable difference of opinion about the post-operative use of anticoagulants. Freeman and his colleagues (Freeman, Wylie and Gilfillan, 1950; Freeman and Gilfillan, 1952) introduced heparin into the affected vessel by means of a polyethylene tube—a procedure they termed *regional heparinization*.

The author uses anticoagulants systemically after thrombo-endarterectomy at any site, and after femoral-popliteal bypass, starting on the day of operation and continuing as a rule for the rest of the patient's life. Heparin is given intravenously every 6 hours for three or four doses. Dindevan (phenylindanedione) is started by mouth on the evening of the day of operation and continued thereafter in a dosage based on regular determinations of the prothrombin time.

Other surgeons prefer not to start giving anticoagulants until some days after operation, and others again do not use anticoagulants at all.

Resection and Replacement of the Descending Thoracic Aorta

We shall consider resection of the descending aorta for a fusiform aneurysm using a bypass technique.

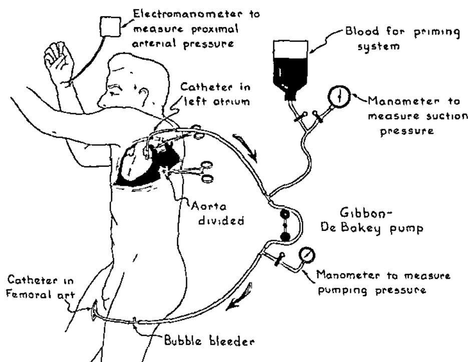


Fig. 151. Shunt with pump from left auricle to femoral artery. (Reproduced from *Surgery* by courtesy of the publishers and Dr. Frank Gerbode.)

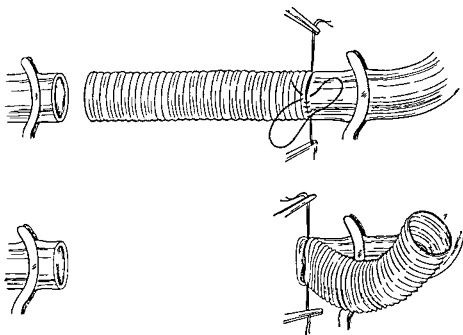


Fig 152 Inserting a flexible prosthesis after resecting an aneurysm of the descending thoracic aorta The proximal anastomosis The anterior half of the suture line is inserted first as shown in the upper drawing The prosthesis is then swung upwards to permit the posterior half of the suture line to be inserted from outside the vessel as shown in the lower drawing

A left lateral thoracotomy is performed through the bed of the fifth sixth or seventh rib or through an interspace, the level depending on the level of the aneurysm The mediastinum is opened and the aneurysm together with healthy aorta above and below is mobilized If the aneurysm is situated fairly low down it may be necessary to incise the diaphragm to facilitate mobilization at the lower end

Heparin is injected intravenously in a dosage of 2 mg /kg body weight A plastic cannula is placed in the left auricular appendage through a purse string suture and connected by polyvinyl tubing through a roller pump to a cannula in the right or left femoral artery as shown in Figure 151 The aorta is clamped above and below using Crafoord or Potts clamps and the pump rate is adjusted to maintain the systolic blood pressure in the lower extremity at a level slightly below that in the arms The aneurysm with a minimal amount of healthy vessel above and below is excised great care being taken not to sacrifice any more intercostal arteries than necessary in view of the danger of paraplegia and a graft or prosthesis is inserted and anastomosed end-to-end to the host aorta with 4/0 silk A continuous over-and-over stitch is used reinforced if it appears necessary by a second tier of sutures The proximal anastomosis is made first (Fig 152) The anterior half of the suture line is inserted and the graft (or prosthesis) is then swung upwards to permit the suture line to be completed from outside the vessel The posterior part of the distal anastomosis (Fig 153) is then made from within the vessel after which the anterior part is completed from outside

If a prosthesis has been used it is wrapped temporarily in polyvinyl alcohol sponge The pump is then stopped and the shunt is dismantled The distal clamp is released first and then if leakage is not excessive, the proximal The aorta is then

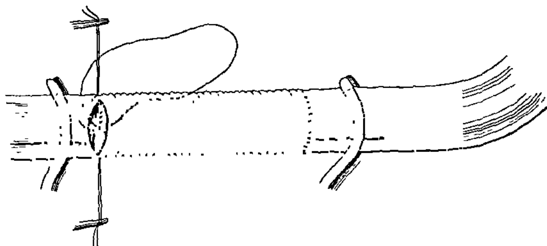


Fig 153. Inserting a flexible prosthesis after resecting an aneurysm of the descending thoracic aorta. The distal anastomosis The posterior half of the suture line is being inserted from within the vessel but the knots are tied on the outside.

compressed manually above the proximal anastomosis as advised by Rob and kept intermittently occluded until the blood pressure becomes stabilized. Protamine is injected and this is repeated four hours later. An intercostal water-seal drain may be inserted through a stab wound before the chest is closed.

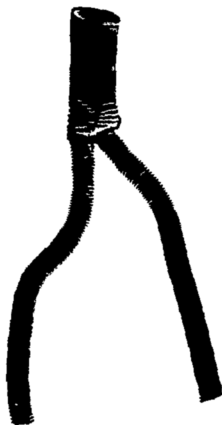


Fig. 154. Aortic bifurcation prosthesis of woven teflon.

Replacement of the Aortic Bifurcation

We shall consider the treatment of Leriche's syndrome (p. 439) using a bifurcation graft or prosthesis.

The abdomen is opened through a long left paramedian incision and the small bowel is either packed off or exteriorized and placed in a plastic bag. The peritoneum over the aortic bifurcation is incised and the aorta and both common iliac arteries are mobilized sufficiently to enable the aorta to be clamped above the block and the iliac arteries below. Care is required not to injure the common iliac veins or the inferior vena cava. Sufficient of the thrombosed segment is trimmed away to enable the graft or prosthesis (Fig. 154) to be placed correctly. The aortic anastomosis is made first in the way described above for the proximal anastomosis after resection of a thoracic aneurysm, after which one iliac anastomosis is made either by an end-to-end technique

or by an end to side technique after closing the end of the host vessel

The remaining steps of the operation differ slightly according to whether a graft or a prosthesis is being used

In the case of a graft the second iliac anastomosis is made in the same way as the first after which first one iliac clamp is released and then the other and if there is no undue leakage the aortic clamp is released The aorta is then intermittently occluded by manual compression until the blood pressure becomes stabilized

If a prosthesis is being used the free limb is clamped the prosthesis is surrounded temporarily by polyvinyl alcohol sponge and the circulation to the opposite extremity is restored by releasing first the iliac and then the aortic clamp The remaining anastomosis is then made (Fig 155) but just before it is completed the clamp on the prosthesis is momentarily relaxed so that any blood clot which has formed is washed out

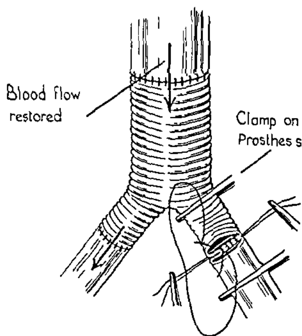


Fig 155 Replacement of the aortic bifurcation by a prosthesis The proximal anastomosis and one of the distal anastomoses have been completed and the blood flow has been restored to the right lower extremity

The abdomen is closed with great care and it is wise to use tension sutures on account of the high incidence of post operative paralytic ileus For the same reason intravenous infusion and gastric suction are maintained for 3 4 days after operation

Occasionally the thrombosis extends up to the level of the renal arteries The operation is then best performed under hypothermia The technique which is more complicated has been described in detail by Rob (1955)

Femoral popliteal Bypass

Femoral popliteal bypass is indicated when there is a segmental block in the superficial femoral artery

The affected limb is externally rotated and the knee is slightly flexed The popliteal artery is exposed through an incision on the medial side of the knee which extends downwards just posterior to the medial border of the tibia as described by McCaughan (1958) The space is opened up between the tibia and knee joint anteriorly and the medial head of the gastrocnemius posteriorly and if necessary part of the origin of this muscle is detached from the medial condyle of the femur and part of the origin of the soleus is detached from the popliteal line of the tibia

When the popliteal artery is exposed it is palpated with the object of determining whether or not it is patent and if there is doubt about this an attempt is made to aspirate blood through a fine needle If even the last 1 2 cm of the vessel is patent the operation presents no difficulty If however the block extends right down to the bifurcation of the popliteal the choice lies between (a) abandoning the idea of doing a bypass operation (b) performing thrombo endarterectomy of the distal part of the popliteal artery before connecting the bypass and (c) attaching the bypass to the posterior tibial artery

or by an end to side technique after closing the end of the host vessel

The remaining steps of the operation differ slightly according to whether a graft or a prosthesis is being used

In the case of a graft the second iliac anastomosis is made in the same way as the first after which first one iliac clamp is released and then the other, and if there is no undue leakage the aortic clamp is released The aorta is then intermittently occluded by manual compression until the blood pressure becomes stabilized

If a prosthesis is being used, the free limb is clamped, the prosthesis is surrounded temporarily by polyvinyl alcohol sponge, and the circulation to the opposite extremity is restored by releasing first the iliac and then the aortic clamp The remaining anastomosis is then made (Fig 155) but just before it is completed the clamp on the prosthesis is momentarily relaxed so that any blood clot which has formed is washed out

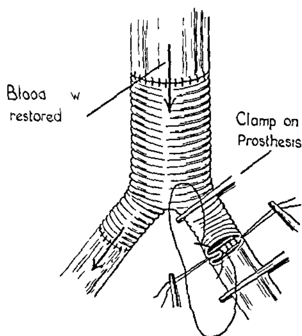


Fig 155 Replacement of the aortic bifurcation by a prosthesis The proximal anastomosis and one of the distal anastomoses have been completed and the blood flow has been restored to the right lower extremity

The abdomen is closed with great care and it is wise to use tension sutures on account of the high incidence of post operative paralytic ileus For the same reason intravenous infusion and gastric suction are maintained for 3 4 days after operation

Occasionally the thrombosis extends up to the level of the renal arteries The operation is then best performed under hypothermia The technique, which is more complicated, has been described in detail by Rob (1955)

Femoral-popliteal Bypass

Femoral popliteal bypass is indicated when there is a segmental block in the superficial femoral artery

The affected limb is externally rotated and the knee is slightly flexed The popliteal artery is exposed through an incision on the medial side of the knee which extends downwards just posterior to the medial border of the tibia, as described by McCaughan (1958) The space is opened up between the tibia and knee joint anteriorly and the medial head of the gastrocnemius posteriorly, and if necessary part of the origin of this muscle is detached from the medial condyle of the femur, and part of the origin of the soleus is detached from the popliteal line of the tibia

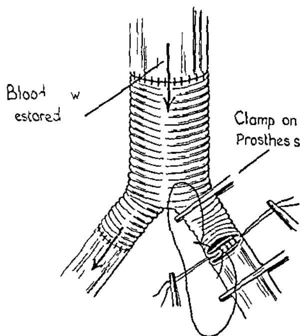
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F g 155 Replacement of the aortic bifurcat on by a prosthes s The prox mal anastomosis and one of the d stal anastomoses have been completed and the blood flow has been restored to the right lo er extrem ty

The abdomen is closed with great care and it is wise to use tension sutures on account of the high incidence of post operative paralytic ileus For the same rea son intravenous infusion and gastric suction are maintained for 3-4 days after operation

Occasionally the thrombosis extends up to the level of the renal arteries The oper ation is then best performed under hypo- thermia The technique which is more complicated has been described in detail by Rob (1955)

Femoral popliteal Bypass

Femoral popliteal bypass is indicated when there is a segmental block in the superficial femoral artery

The affected limb is externally rotated *and the knee is slightly flexed* The pop liteal artery is exposed through an incision on the medial side of the knee which ex tends downwards just posterior to the medial border of the tibia as described by McCaughan (1958) The space is opened up between the tibia and knee joint anter iorly and the medial head of the gastro- cnemius posteriorly and if necessary part of the origin of this muscle is detached from the medial condyle of the femur and part of the origin of the soleus is detached from the popliteal line of the tibia

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or by an end to-side technique after closing the end of the host vessel

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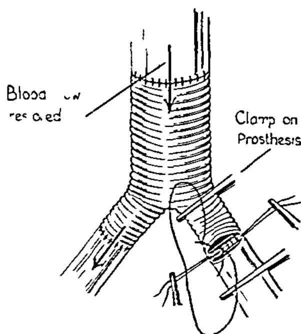


Fig 155 Replacement of the aortic bifurcation by a prosthesis. The proximal anastomosis and one of the distal anastomoses have been completed and the blood flow has been restored to the right lower extremity

The abdomen is closed with great care and it is wise to use tension sutures on account of the high incidence of post operative paralytic ileus For the same reason intravenous infusion and gastric suction are maintained for 3-4 days after operation

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Femoral popliteal bypass is indicated when there is a segmental block in the superficial femoral artery

The affected limb is externally rotated and the knee is slightly flexed The popliteal artery is exposed through an incision on the medial side of the knee which extends downwards just posterior to the medial border of the tibia as described by McCaughan (1958) The space is opened up between the tibia and knee joint anteriorly and the medial head of the gastrocnemius posteriorly, and if necessary part of the origin of this muscle is detached from the medial condyle of the femur, and part of the origin of the soleus is detached from the popliteal line of the tibia

When the popliteal artery is exposed it is palpated with the object of determining whether or not it is patent and if there is doubt about this an attempt is made to aspirate blood through a fine needle If even the last 1 2 cm of the vessel is patent the operation presents no difficulty If however the block extends right down to the bifurcation of the popliteal the choice lies between (a) abandoning the idea of doing a bypass operation (b) performing thrombo-endarterectomy of the distal part of the popliteal artery before connecting the bypass and (c) attaching the bypass to the posterior tibial artery

The common femoral artery and the upper part of the superficial femoral are approached through an oblique incision just below the inguinal ligament. A tunnel is formed extending from the femoral triangle to the popliteal fossa. It is started by finger dissection at each end and completed with an oesophagoscope (as suggested by Rob) or, more simply, with an instrument of similar calibre fitted with an obturator.

The author prefers to use either a homograft or a crimped teflon prosthesis. Nylon, though still used by some surgeons, is much less satisfactory; and dacron, though better than nylon, does not seem to be as satisfactory as teflon. The usual size of prosthesis is 5/16 inch diameter. This admittedly is of considerably greater calibre than the popliteal artery but has been found to be the most suitable size for end-to-side anastomosis to this vessel. The method of preparing the prosthesis for use has already been described (p. 446).

The host vessel is controlled with tape tourniquets (*see* Fig. 144), and as soon as these have been applied heparin (30 mg.) is injected distally into the popliteal artery. The lower anastomosis is made end-to-side by inserting two pairs of stay sutures and joining them by an over-and-over stitch (*see* Fig. 14). The free end of the graft or prosthesis is then drawn upwards with long forceps through the instrument used to make the tunnel, after which the instrument is withdrawn. The upper anastomosis is made in the same manner as the lower one, while the graft or prosthesis is held under moderate tension. It is the author's practice to suture each wound but to leave in a soft rubber (Penrose) drain, and to give anticoagulants postoperatively from the day of operation (p. 447), but as we have seen some surgeons do not use any anticoagulants at all and others do not start anticoagulant therapy until several days after the operation.

CHAPTER 22

Transplantation of Haemopoietic Tissue

In recent years a great deal of work has been done on the transplantation of haemopoietic tissue stimulated for the most part by the need for developing methods of combating the harmful effects of irradiation (*see* Congdon 1957 *Blood* 1957)

The reasons why this need has become so compelling are not far to seek In the first place the risk of accidental exposure to ionizing radiation in high dosage both in peace and in war has enormously increased Secondly it seems likely that the scope and value of radiotherapy in the treatment of neoplastic disease would be increased if means could be found of increasing the dosage without detriment to the patient Finally the whole question of the biological effects of irradiation is becoming of ever increasing interest and importance

As we have seen (p 99) death following total body irradiation to or a little beyond the minimum lethal dosage is usually the result of damage to either the haemopoietic tissues or the gut and which factor predominates depends on the species of animal concerned and on the physical characteristics of the radiation

Various methods have been used to protect against or counteract the effects of radiation and these may be divided into three groups according to whether they have to be applied before during or after exposure One of the methods used after exposure is transplantation of haemopoietic tissue and it is this which forms the subject of the present chapter

We shall however not confine our attention to transplantation following irradiation but will consider also transplantation in both animals and man following administration of substances such as nitrogen mustard and Myleran which cause widespread damage to haemopoietic tissue resembling in many ways the damage caused by irradiation and in patients suffering from aplastic anaemia and agammaglobulinaemia

HISTORY

ANIMAL EXPERIMENTS

Auto-, Iso, and Homo-transplantation Following Total Body Irradiation in Rodents

Protective Effect of Transplantation

Talbot and Gerstner (1951) reported that the mean survival time of rats injected intravenously with homologous marrow after irradiation to 800r was not significantly longer than that of irradiated but non injected controls Lorenz Uphoff Reid and Shelton (1951) on the other hand found that intravenous or intraperitoneal injection of isologous marrow to irradiated inbred mice or guinea pigs had a marked protective effect and they suggested that this was probably due to persistence and

multiplication of the injected cells because foci of haemopoietic tissue were found in the liver; they considered however that a humoral factor might also play a part. Later Lorenz and his colleagues (Lorenz, Congdon and Uphoff, 1952; Congdon, Uphoff and Lorenz, 1952) confirmed and extended these findings. They showed *inter alia* that intravenous injection of homologous, or even of heterologous (guinea pig), marrow had some protective effect in mice irradiated to 900r (a dose which was normally lethal in 21 days). In view of the effect of heterologous cells they revised the opinion expressed previously and suggested that the protective effect was probably due mainly to a humoral factor, though a cellular factor might play some part also. When the protection was complete the animal's haemopoietic tissue had fully recovered at the end of 21 days. The total period of observation following irradiation ranged from 30 to 60 days, and it was observed that some animals died after the 21st day. The cause of death was obscure but was described as being "*not due* to exhaustion of haemopoietic tissue." The possible significance of these deaths will be considered later (p. 457). Lorenz and his colleagues suggested that the failures reported previously might have occurred because insufficient precautions were taken to ensure that the injected cells were viable.

Holub (1951) studied the effect of transplanting homologously the diaphysis of a long bone to rabbits and rats which had been subjected to total body irradiation in a dosage of 300r. He found that the leucocyte count in the peripheral blood began to rise after 5-14 days, and that the mortality was decidedly less in these animals than in controls which were irradiated but did not receive a graft. He believed that the marrow in the grafts was destroyed, but that some substance was liberated from the bone which stimulated the formation of new marrow of host origin. In support of this hypothesis Holub cited experiments by Rohlich in which areas of haemopoiesis appeared in the course of a few months around grafts not only of fresh marrow but also of marrow which had been killed by heat or chemicals, and later reported experiments of his own (Holub, 1953) in which heterografts of bone from which the marrow had as far as possible been extracted also apparently evoked new marrow formation.

Jacobson, Simmons, Marks, Gaston, Robson and Eldredge (1951) reported that the mortality in heavily irradiated mice could be reduced by subsequent intraperitoneal isotransplantation of splenic tissue from infant mice, and they confirmed the previous observation of Jacobson and his colleagues (Jacobson, Marks, Gaston, Robson and Zirkle, 1949; Jacobson, Marks, Robson, Gaston and Zirkle, 1949) that shielding the spleen of a mouse during exposure to radiation had a protective effect. Jacobson and his colleagues (*op. cit.*; Jacobson, Simmons, Marks and Eldredge, 1951) believed that the protective effect in both kinds of experiments was probably humoral in origin, but they did not entirely rule out the possibility that recovery of the irradiated tissue was due in part to cells migrating from the transplants or the shielded tissue as the case might be. A striking feature of the spleen-shielding experiments was that some protection was obtained even if the spleen was subsequently removed, provided that it was left in place with an intact circulation for at least an hour after the irradiation. If, therefore, one accepts the cell migration hypothesis one is faced with the question of whether there is normally a continuous circulation of primitive haemopoietic cells from the spleen or, alternatively, whether the migration is stimulated by the trauma of the shielding operation or by chemical factors liberated from the non-shielded tissues.

Barnes and Loutit (1953) confirmed the work of Jacobson, Simmons, M

ton Robson and Eldredge (1951) in a qualitative sense though their findings were less striking. They also obtained protection with intravenous injection of minced spleen but not with injection of the supernatant fluid obtained by centrifuging this material and they therefore suggested that either there was no humoral agent, or it was present only in very low concentration.

Cole and his colleagues (Cole, Fishler and Bond 1953; Cole and Ellis 1955) found that homogenates of spleen in which the tissue had been subjected to severe trauma were just as effective as cell suspensions in protecting from irradiation injury. Differential centrifugation of these homogenates showed that the active fraction was the heaviest and that it consisted almost wholly of nuclei and was destroyed by desoxyribonuclease and by trypsin. They suggested therefore that the active principle was macromolecular particulate and probably desoxyribonucleoprotein.

At this stage then the nature of the protective agent was uncertain but it was not long before further evidence was obtained which pointed strongly to its being after all dependent on survival of the transplanted cells. Barnes and Loutit (1954) showed that prior administration of homologous cells to elicit an immune response prevented the therapeutic effect of a second post-irradiation injection of identical material. Lindsley and his colleagues (Lindsley, Odell and Tausche 1955; Odell, Tausche, Lindsley and Owen 1957) were able to identify two types of erythrocytes, one type with antigens derived from the donor and the other with antigens derived from the host in the peripheral blood of irradiated rats which had received homologous bone marrow. Mitchison (1956) irradiated CBA mice to 950r and then immediately injected approximately 15 million spleen cells from 8 day old A mice. The spleen, lymph nodes and peritoneal exudate of the host animals, obtained at intervals ranging from 4 to 51 days after irradiation, were proved to contain A antigen by demonstrating their capacity to immunize CBA mice against an A strain tumour. Furthermore irradiated mice which received isologous or homologous spleen cells from mice immunized against a bacterial antigen subsequently showed a much higher titre of antibody than non-irradiated hosts which received similar cell injections.

The evidence cited in the last paragraph is cumulatively impressive but it is not quite decisive and some of the experimental findings could be explained by postulating that antigen is transferred from donor cells to host cells by a process akin to transduction in bacteria. Ford, Hamerton, Barnes and Loutit (1956) therefore carried out further experiments using as donors mice of a strain (T6) possessing a characteristic chromosome which could be recognized easily in Feulgen squash preparations of marrow cells previously treated with colchicine and could therefore be used as a 'marker'. By this means they were able to demonstrate donor cells in the marrow of irradiated hosts 5, 11, 14, 19, 28 and 49 days after intravenous injection (*see also* Ford 1956) and these animals could therefore properly be termed chimeras (Fig. 156).

Subsequent investigations have shown that provided the irradiation is sufficient to reduce the marrow count to a low level and the dose of injected cells is large the process of recolonization is extremely rapid. Thus Urso and Congdon (1957) observed a rise in the marrow cell count a few minutes after intravenous injection of isologous cells to mice which had been irradiated to 900r three days previously, and Congdon (1958b) has stated that homologous cells reach the host marrow equally quickly. Moreover, if the animal survives the state of chimerism may persist without any evidence of regeneration of host marrow for more than a year (Ford, Ilbery and Loutit, 1957) and probably indefinitely.

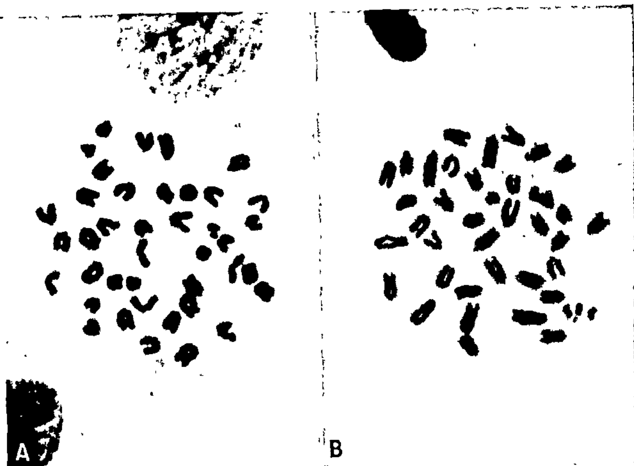


Fig. 156 Cytological identification of radiation chimeras by means of Feulgen squash preparations following colchicine injection. ×3660. A. Chromosomes (40) of a cell from the spleen of a normal mouse The chromosomes vary in length by a factor of a little more than 2 and all have almost terminal centromeres. B. Chromosomes of a T6/4 cell from the thymus of a T6/4 (donor)—CBA (host) chimera The T6 marker chromosome is about half the length of the next shortest (normal) chromosome It is on the edge at 4 o'clock. (Dr. C. E Ford's specimen)

In rabbits polymorphonuclear leucocytes from female donors can be recognized cytologically (*see* p. 459). Porter (1957) showed by this test that donor polymorphs were present in the peripheral blood within 3-4 days of irradiation (800-1000r) in a proportion of animals, and rapidly increased in number until the total leucocyte count became normal or even higher than normal.

The number and nature of the cells injected are important factors in experiments of the kind we have been considering.

When isologous cells are used the number required is astonishingly small. Jacobson, Marks and Gaston (1955) estimated that 50,000 cells injected intravenously sufficed to produce significant recovery in mice irradiated to LD 99.*

Urso and Congdon (1957) investigated the matter in more detail and studied the effect of eight different cell dose levels (ranging from 7,000 to 240 million nucleated cells)† on the general appearance and behaviour, mortality, body weight, bone marrow response, blood leucocyte count, spleen weight, and thymus weight of mice irradiated to 920r and injected intra-

*i e. the dose of irradiation corresponding to a mortality of 99 per cent.

†Here and subsequently we quote only the first two significant figures of the cell counts recorded by Urso and Congdon

venously with isologous marrow cells immediately afterwards They found that the marrow response provided the first evidence of recovery The marrow cell count fell after the irradiation and with a dose of 16 000 cells or less it invariably continued to fall until the animal died With higher doses the marrow count began to rise after 2 6 days and reached a maximum after 4 14 days the recovery being quickest when the dose was greatest The blood leucocyte count usually began to rise 1 2 days after the first evidence of recovery in the marrow and the maximal response was attained at a dose of 64 million cells The rate of recovery of the normal weight of the spleen thymus and whole animal also showed a direct correlation with the cell dose but maximal responses were attained at 13 million 64 million and 13 million cells respectively Animals which received less than 64 million cells showed ruffling of hair and reduced activity for a period related inversely to the dosage Optimal 30 day survival was reached with a dose of 64 million cells The doses calculated to give 50 per cent and 1 per cent survival were 420 000 and 8 000 respectively for males and 1 million and 10 000 respectively for females but the 95 per cent confidence limits were so large that the authors did not claim that these estimates were reliable

In mice at any rate isologous marrow cells and isologous spleen cells seem to be about equally effective (*see* e g van Bekkum 1958) Intravenous injection is the most effective method of administration but intraperitoneal injection is effective if somewhat larger doses are used

When homologous cells are used much larger doses are required Van Bekkum (1958) for example found in mice that 100 million homologous marrow or spleen cells gave about the same degree of protection as 100 000 isologous cells The reason for this difference is not clear and merits investigation

The fact that the dose of haemopoietic cells required to protect lethally irradiated animals depends on whether the cells are isologous homologous or heterologous makes it possible to study the fate of the transplanted cells by a retransplantation test The principle of this test is that measured doses of cells from the marrow of an animal A which has been irradiated and repopulated with homologous or heterologous haemopoietic tissue from a donor B are injected into lethally irradiated animals C isogenic with A If the minimal dose required to protect C is found to be about what would be required with isologous cells it may be argued that A s own marrow has largely recovered although other explanations can be suggested *(v infra)* If on the other hand the dose required is significantly larger than this it is concluded that A s marrow contains at least a proportion of B s cells

This test has been exploited by Vos Davids Weyzen and van Bekkum (1956) and Urso McKinley and Congdon (1958) Their observations suggest that the proportion of host cells in the bone marrow of lethally irradiated mice given homologous cells is very small 30-40 days after grafting but by 60 days is high enough to give an isologous survival response It is difficult however to reconcile this with the cytological studies of Ford Ilbery and Loutit (p 454) in which there was no evidence of regeneration of host marrow more than a year after irradiation and homotransplantation and Urso *et al* have therefore suggested that their findings may have been due not to recovery of host marrow but either to some alteration in the antigenicity of the donor marrow because of prolonged contact with the foreign host or to the donor cells becoming tolerant of the host

Combined Methods of Protection

As already mentioned death following irradiation at or a little above the mini

mum lethal dose may result from either damage to haemopoietic tissue or peritonitis, and at higher dosage potentially lethal damage may occur in many organs. Some interesting observations have been made on the effect of combining different methods of protection, including administration of sulphydril compounds such as AET (S, 2-aminoethylisothiuronium . Br . HBr) and MEG (2-mercaptoethylguanidine . HBr)* before or during irradiation, and transplantation of haemopoietic tissue and administration of antibiotics after irradiation. Congdon (1958b), for example, found in one strain of mice that the LD 50 (30 days) was 700r if no treatment was given, 1100-1200r if isologous marrow was given after irradiation, 1400r if AET was given before irradiation, and 1800r if AET was given before irradiation and isologous marrow afterwards. When AET was given before irradiation, and both isologous marrow and streptomycin afterwards, some animals survived a dose of 2,500r.

Secondary Disease

Barnes and Loutit (1954) drew attention to the fact that irradiated (CBA) mice which received homologous (A) spleen cells survived for shorter periods than those which received isologous cells. Later Barnes, Corp, Loutit and Neal (1956) succeeded in curing leukaemia in mice by irradiation and injection of isologous bone marrow cells,† but found that if homologous marrow was used instead, after the same dose of irradiation (1,500 rads in 24 hours), the recipients developed a peculiar syndrome characterized by marked loss of weight and diarrhoea, and died 1-2 months after treatment despite the fact that the leukaemia appeared to have been eradicated. This syndrome, which is now usually referred to as *secondary disease*, has since been observed by many investigators in animals which have received homologous spleen cells following total body irradiation and may develop within two weeks or even less. It has been confirmed that secondary disease may also occur after irradiation and injection of homologous marrow (Congdon and Urso, 1957), but it then usually takes longer to develop, runs a milder course and carries a much lower mortality (van Bekkum and Vos, 1957; Schwartz, Upton and Congdon, 1957). At autopsy widespread destruction of lymphoid tissue similar to that found in "runt disease" (p. 125) is seen. According to Congdon (1958a) corticosteroids, antihistamines, antibiotics and vitamins do not affect the outcome, but the syndrome is less likely to develop if the marrow injection is delayed for 1-3 days after the irradiation.

Secondary disease is not confined to mice but has been studied most thoroughly in this species and has been found to be more severe with certain strain combinations, notably when the donor and host are of different H2 genotype. Indeed, as Uphoff and Law (1958) have shown, when spleen cells are used a difference in a single histocompatibility gene at the H2 locus may suffice to ensure a severe form of the condition.

These features, together with the fact that secondary disease does not occur when isologous cells are used, suggest that it is a manifestation of an immunological reaction of the foreign cells against the host. Though some investigators have been reluctant to accept this hypothesis, the further discovery that the syndrome does not occur following injection of marrow from F1 hybrids of two inbred strains to either irradiated F1 hybrids (Uphoff, 1957) or irradiated animals of either parent strain (Trentin, 1958), but does occur with most strain combinations* when cells from one

*For a discussion of the effect of these agents see Overman (1958b) and Doherty (1958).

†Cure of experimental leukaemia by irradiation and marrow transplantation has been reported also by Lorenz and Congdon (1955).

*With some strains bone marrow from a parent strain to an irradiated F1 causes little or no secondary disease. Possible reasons for this will be considered later (p. 459).

of the parent strains are transplanted to irradiated F1 hybrids (Schwartz Upton and Congdon 1957 Trentin 1957b Uphoff 1957 Ilbery Koller and Loutit 1958) provides strong evidence in its favour

For reasons discussed in Chapter 7 it would tell strongly in favour of the graft against host hypothesis if it could be shown that homologous haemopoietic cells from sufficiently immature donors protected irradiated hosts without causing secondary disease This has been investigated by injecting irradiated animals with cell suspensions prepared from homologous foetal liver because in rodents even at the time of birth haemopoiesis occurs mainly in the liver

The first experiments of this kind which were performed by Lacassagne Duplan and Buu Hoi (1955) in rats were unsuccessful and the recipients did not survive long possibly because the number of cells injected was too small *More recently however* further investigations have been carried out in mice and rabbits

In mice Uphoff (1958a) found that irradiated (C57BL x DBA/2) F1 hybrids recovered completely and showed no manifestations of secondary disease during a 90 day period of observation following injection of 37 million nucleated cells obtained by mincing the liver and spleen of C57BL foetuses whereas there was a high mortality from secondary disease following injection of bone marrow from C57BL adults Furthermore irradiated AKR mice which received homologous C3H foetal liver survived longer than those which received C3H adult marrow

Further evidence of the advantage of foetal over adult haemopoietic tissue as far as the avoidance of secondary disease in mice is concerned has been obtained by Urso (1958) Lengerová (1958) Barnes Ilbery and Loutit (1958) and Urso Congdon and Owen (1958) Urso Congdon and Owen showed not only that the outward manifestations of secondary disease were avoided in their experiments when foetal liver was used despite the fact that donor type cells could be demonstrated in the recipient's blood but also that only very mild changes occurred in the lymph nodes as compared with those observed in irradiated animals given adult homologous marrow They reported further that spleen or liver from one day old mice was as effective (and innocuous) as foetal tissue but tissue from 2 6 day old mice caused a high incidence of secondary disease

It has been reported that secondary disease occurs with certain strain combinations even when foetal liver is used (Owen and Urso 1958 Uphoff 1958b) The diagnosis has sometimes been made however merely on the basis of a delayed drop in body weight and without further information it is not possible to decide whether or not the condition resulted simply from late *rejection of the graft*

In rabbits Porter and Moseley (1958) injected irradiated adults with cell suspensions prepared from the livers of new born donors Four out of 10 animals which were irradiated to 900r and then received 500 million nucleated cells survived for the duration of the experiment (56 days or longer) Five died from either peritonitis or marrow aplasia associated with rejection of the transplanted cells but one died 11 days after irradiation with signs of secondary disease including diarrhoea and atrophy of lymphoid tissue This suggests that the new born rabbit is nearly but not quite immature enough to provide cells which are incapable of mediating a graft against host reaction but further evidence is required *to put the matter beyond doubt*

The Cells Responsible for Protection and Secondary Disease

It appears from the evidence cited that adult spleen adult bone marrow and foetal liver are each capable of giving rise to all the types of cell normally found in the peri

pheral blood. Whether these cells arise from one single stem cell is an unresolved question which has exercised the dialectical talents of haematologists for many years. Further experiments on the repopulation of irradiated animals with isologous cells might throw considerable light on the matter if satisfactory isologous markers were available. In some species chromatin sexing (Barr and Bertram, 1949; *see* also Barr, 1956; Lennox, 1956) could be used to distinguish polymorphs of donor origin following transplantation from females to irradiated males, but unfortunately in rats and mice male and female cells cannot be distinguished in this way, and it is not possible to distinguish between male and female lymphocytes in any species.

In the absence of an isologous marker little progress can be made because there is always the possibility of some types of cell being formed from regenerating host tissue. Thus, for example, Smith and Congdon (1957) found that lethally irradiated BALB/C mice recovered following injection of 100 million isologous leucocytes from leukemoid blood, the vast majority of which were granulocytes, but they were uncertain whether the transplanted cells provided complete replacement or, by providing an adequate supply of granulocytes, protected the host against infection and prolonged its life sufficiently for regeneration of other elements to occur.

A simpler question is whether repopulation can be achieved more quickly by using both bone marrow and lymphoid tissue rather than either one alone. The answer is suggested by the observation of Congdon, Makinodan, Gengozian, Shekarchi and Urso (1958) that lymphoid tissue in irradiated mice recovers more quickly after injection of isologous spleen cells than after injection of isologous marrow, but much more work is needed to determine the optimal combination of cells for isologous replacement.

When homologous cells are used the position is complicated by the risk of secondary disease. As we have seen spleen cells in equal dosage appear to be much more harmful than marrow cells, and it seems likely in view of the known capacity of lymphocytes to cause runt disease (p. 125) that this is due to the large proportion of lymphocytes in splenic tissue. Marrow from donors previously immunized with recipient strain tissue is more productive of secondary disease than normal marrow (Schwartz, Upton and Congdon, 1957), but it remains to be seen whether this is due simply to a change in the immunological status of cells normally resident in the marrow, or to a change in cell population and in particular an increase in the proportion of lymphocytes. Conversely Uphoff (1958c) has found that secondary disease may be avoided if a folic acid antagonist, methopterin, is administered together with homologous marrow to irradiated recipients, but again the mechanism involved has not yet been established.

Auto-, Iso-, and Homo-transplantation Following Total Body Irradiation in Large Animals

Dogs

Rekers, Coulter and Warren (1950) exposed dogs to 350r total body irradiation and then treated them by intramedullary, intravenous, intrasplenic or intrahepatic injection of a cell suspension made from homologous bone marrow, by orthotopic homotransplantation of bone and marrow, by homotransplantation of minced whole embryos to the abdominal wall and the anterior chamber of the eye, or by heterotransplantation of minced whole beef embryos to the abdominal wall and the anterior chamber of the eye. They found that intravenous injection of approximately 20,000 million homologous marrow cells caused improvement in the blood picture and reduced the mortality from 67 per cent to 43

per cent whereas the other procedures were ineffective

Ferrebee, Lochte, Jaretzki, Sahler and Thomas (1958) used four healthy young beagles which had been de-wormed and given distemper and hepatitis vaccine Two of the prospective hosts were splenectomized and all were given a course of ACTH (10 i u twice daily) for 24 30 days They were then irradiated in several positions on three successive days with a 250 kv machine, the total dose being 1200r (approximately three times the LD 50) After irradiation the animals received an intravenous injection of 8 000 million to 10,000 million nucleated marrow cells from a beagle of the opposite sex Then they were given penicillin streptomycin oxytetracycline and ACTH and a diet of milk and strained liver In some animals the peripheral blood leucocyte count, after a sharp fall gradually rose to its original level or even higher and some at least of the leucocytes appeared to be female in type When antibiotics and ACTH were withdrawn however the animals temperature rose and the leucocyte count fell sharply One animal which had received marrow from a sibling eventually recovered completely but this appeared to be due to regeneration of its own marrow These findings are consistent with the view that a graft against host reaction occurred but was held in check for a time by the administration of ACTH

Egdahl and Hume (personal communication) exposed mongrel dogs weighing approximately 20 kg to 600r using a 1 MeV linear accelerator The irradiation was given in a single dose over a period of about 20 minutes Twelve hours after irradiation the animals received an intravenous injection of a suspension of homologous foetal liver from foetuses of 4 8 weeks gestation Two recipients survived They were reported to be well and to show a normal peripheral blood picture 2¼ and 5 months respectively after irradiation

Pigs

Akeroyd (1958) exposed pigs to atomic radiation and then injected homologous marrow and spleen cells to the vena cava or the peritoneal cavity, but all the animals died

Monkeys

Overman (1958a, b) found that the LD 100 for rhesus or *Macacus mulatta* monkeys using a 250 kv machine, was 600-625r Forty animals were given 650 700r, and about half of them received no other treatment and died within 17 days The remainder each received an intravenous injection of 1,000 million to 2,000 million nucleated marrow cells from a young monkey of the same species 24-48 hours after the irradiation, and 60 per cent of them survived more than 30 days, by which time the leucocyte count in the peripheral blood which had fallen sharply and had reached a minimum about 14 days after irradiation had returned to normal Some of these animals were sacrificed soon afterwards, one died after 167 days showing emaciation and other features characteristic of secondary disease (p 457) and some were still alive and well 174 days after irradiation In another experiment 8 monkeys received 800 900r irradiation followed by intravenous injection of 1 500 million homologous marrow cells All died, but in 6 animals the blood picture appeared to be returning to normal and death was attributed to damage to the gastro-intestinal tract

Auto-, Iso-, and Homo-transplantation Following Administration of Marrow Poisons

As we have seen (p 100) various compounds have been found which cause widespread damage to haemopoietic tissue resembling in many ways the damage caused by irradiation

Weston and his colleagues (Maxwell and Weston, 1956, Weston, Maxwell, Lee, Finzel

and Fiskell, 1957; Weston, 1958) used one such compound, Myleran, in rats and then investigated the effect of injecting homologous marrow cells. The animals each received 20 mg. Myleran per kg. body weight intravenously. In the absence of treatment the nucleated cell count in the marrow 3-4 days later was 250,000 cells/c.mm. of which 90 per cent were lymphocytes, and within 14 days 90 per cent of the animals were dead. On the other hand animals which received a single injection of 125 million homologous marrow cells 1-5 days after the Myleran showed striking marrow regeneration, clearly recognizable after about 11 days, and many of them were alive and well (apart from a high incidence of cataract) a year later. Repeated daily doses of 125 million cells were no more effective than a single dose, and Weston concluded in consequence that cell repopulation might not be the only explanation and that there might be a humoral factor as well, but his argument is unconvincing.

Heterotransplantation

Polymorphonuclear leucocytes giving the alkaline phosphatase reaction characteristic of rat cells were found by Nowell and his colleagues (Nowell, Cole, Roan and Habermeyer, 1957; Nowell, Cole, Habermeyer and Roan, 1956; *see* also Cole, Habermeyer and Nowell, 1957), and also by Makinodan (1956) and Gengozian and Makinodan (1956), in irradiated mice which had been injected with rat marrow, but, as Nowell *et al.* pointed out, this might conceivably have been due to transformation of the host's own cells. Proof of survival was obtained however by Ford *et al.* (1956), who were able to identify numerous rat cells, but no mouse cells, in Feulgen squash preparations of colchicine treated cells from irradiated mice which had been injected with rat marrow.

In addition to leucocytes, red cells having the serological properties of rat red cells and containing haemoglobin having the chemical properties of rat haemoglobin (Makinodan and Anderson, 1957), and blood platelets having the serological properties of rat platelets (Smith, Makinodan and Congdon, 1957; Repplinger, Schwartz, Congdon and Tocantins, 1958), have been demonstrated in the blood of irradiated mice which received rat marrow.

So far recolonization has not been achieved with heterologous spleen cells, and has only been achieved with heterologous marrow when the donor and host belonged to fairly closely related species such as the rat and the mouse, or the guinea pig and the mouse. The number of cells required to be injected is much larger than when homologous cells are used, and the dose of irradiation may have to be well above the LD 100, the precise level depending on the relationship between donor and host species. If the graft survives the recipient is likely to develop a severe form of secondary disease, but a proportion, which varies greatly according to the conditions of the experiment, recover and become permanent hetero-chimeras. Thus according to Makinodan and Anderson (1957) mice which receive rat marrow after irradiation to 600-675r occasionally become permanent chimeras and, as Welling, Vos and van Bekkum (1959) have shown, those which receive rat marrow after irradiation to 800r commonly do so, but according to Makinodan (1958) the dose has to be increased to 1000-1100r before they will accept guinea pig marrow. Foetal and new-born haemopoietic tissue appears to have no advantage over adult tissue for heterotransplantation, and Urso, Congdon and Owen (1959) found that even after irradiation to 950r only a few mice which received liver from foetal or new-born rats became permanent chimeras, and the 30-day survival was no better than with similarly irradiated animals which received the same number of adult rat bone marrow cells.

Weyzen and Vos (1957) showed by a pre cipitin test that the serum of mice which had received rat marrow after irradiation con tained rat protein Subsequently Grabar Courcon Merrill Ilbery and Loutit (1958) showed by immuno electrophoresis (p 88) that the serum of mice which developed sec ondary disease following irradiation and transplantation of rat marrow contained rat gamma globulin They concluded that the secondary disease was due either to a graft against host reaction or to a weakening of the animal s normal immune defen e aga nst its own bacterial flora due somehow to the presence of foreign gamma globulin

Transplantation of Other Tissues to Irradiation Chimeras

As we have seen (p 137) Main and Prehn (1955) and Trentin (1956 195) found that homografts of skin from mice of one strain survived in mice of another strain which had been irradiated and then injected with bone marrow from a hybrid of the two strains or a member of a different strain More recently St Amand and Smith (per sonal communication) transplanted ovaries orthotopically from A mice to irradiated (950r (10 ×C3H) F1 females and obtained albino young when these animals were mated with albino males

As might be expected this tolerance ap pears to be at least to some extent specific but it remains to be seen whether there is also some degree of non specific tolerance to homografts from other donors

It ha been shown further that irradiated mice which have been repopulated with rat bone marrow will tolerate heterografts of rat skin so long as the state of chimerism persists (Zaalberg Vos and van Bekkum 1957)

The General Immunological Status of Irradiation Chimeras

Mice which recover from secondary dis ease after lethal irradiation and homotrans plantation or heterotransplantation of bone marrow may after 10 weeks or so look very like similarly irradiated animals treated with isologous marrow but their capacity to re act to antigens may be very different

The matter was investigated by Gengo zian Makinodan Congdon and Owen (1958) They found that mice repopulated with homologous marrow 200 300 days pre viously showed a subnormal response to rat erythrocytes and an even more depressed response to sheep erythrocytes Mice which had been repopulated with rat marrow and were permanent hetero chimeras showed an extremely poor response to sheep erythro cytes and 3 of 12 animals failed to produce detectable antibody

Further experimental evidence is needed before the general significance of these find ings can be assessed but they suggest that there may be a considerable non specific element in the capacity of irradiation chi meras to accept grafts of other tissues

The Life Span of Irradiation Chimeras

Hollaender Congdon Doherty Makino-dan and Upton (1959) compared *inter alia* the cumulative mortality curve of mice sur viving 30 days or longer after (a) irradiation 700 800r (b) irradiation 1100 1200r fol lowed by injection of isologous marrow cells (c) irradiation 1200 1400r and admin istration of AET (p 457) (d) irradiation 1600 1800r together with administration of AET and injection of homologous marrow cells The mortality at corresponding times in (b) (c) and (d) was only slightly higher than in (a) and Hollaender *et al* therefore suggested that AET and bone marrow both protect against the delayed life shortening effect of irradiation Further analysis showed that both markedly inhibit the de velopment of leukaemia but have little or no effect on the incidence of nephroscler-osis which becomes the main cause of death

Further evidence of the leukaemia-inhibiting effect of bone marrow transplants was obtained by Law and Uphoff (personal communication) who showed that mice which received four doses of irradiation, each of 168r, at seven day intervals and were then given isologous marrow had a lower incidence of leukaemia than similarly irradiated mice which were not given any marrow.

CLINICAL OBSERVATIONS

Autotransplantation

In two patients with widely disseminated neoplastic disease Kurnick, Montano, Gerdes and Feder (1958) collected bone marrow prior to irradiation and replaced it afterwards as an autotransplant.

The first patient had pulmonary metastases from a malignant tumour of the testis. Bone marrow was aspirated from the ilium and sternum into Tyrode's solution containing 30 per cent glycerol, to which was added 5 units heparin/ml. marrow. The suspension was frozen in a Barnes-Loutit apparatus (p. 468) and stored in a dry-ice cabinet. The patient was irradiated repeatedly with a 400 Kv. machine, the average tissue dose to the entire torso over a period of two months being 200r. The patient received intravenous infusions of marrow on two occasions, the estimated number of viable nucleated cells injected being 400 million and 585 million respectively. The method of thawing and subsequent treatment are described in the section on storage (p. 468). The proportion of viable cells was estimated by the unstained cell count method of Schrek (p. 86) to be between 75 and 80 per cent of the total nucleated cells. Bone marrow smears showed evidence of severe marrow hypoplasia immediately after irradiation with rapid recovery following the marrow infusions. The patient improved considerably for about a month, but then began to fail rapidly due to progress of the metastases and died six weeks after the first marrow infusion. Bone marrow obtained from the lumbar vertebrae at autopsy appeared to be cytologically normal.

The second patient, who was suffering from disseminated cancer of the kidney, was treated along similar lines, but failed to improve and died ten days after the commencement of treatment.

More recently Newton, Humble, Wilson, Pegg and Skinner (1959) have combined autotransplantation of bone marrow with high dosage supervoltage X-ray therapy to the chest in two patients with pulmonary metastases from malignant bone tumours, with encouraging results in one case.

Homotransplantation without Prior Irradiation or Administration of Marrow Poisons

According to Migdalska (1958), J Raszek of Lwow attempted to treat lymphatic leukaemia and pernicious anaemia by transplantation of bone and marrow as long ago as 1939, and in 1952 Migdalska himself reported that he had treated 30 patients suffering from thrombocytopenic purpura and other haemorrhagic diseases with homologous marrow and had obtained some good results. It is hardly conceivable, however, that the injected cells escaped the usual fate of homografts.

Aplastic anaemia appeared to offer a more fruitful field for investigation because it seemed possible that the host's immunological competence would be drastically curtailed as a consequence of his disease.* The matter was investigated by Dameshek and his colleagues (cited by McFarland, 1958), who gave repeated marrow transfusions to patients with aplastic anaemia. The cell dosage was large, each patient receiving 1,000 million to 2,000 million cells from each of 8-12 donors, and large doses of corticosteroids were [illegible] in addition.

*On [illegible] as Betman (1958) has pointed out, [illegible] patient with aplastic anaemia is [illegible] nown reason, to support the [illegible] ssue whatever its origin.

Initially the results were disappointing and at best could only be described as equivocal (McFarland 1958) but later Dameshek Mc Farland and Granville (1958) reported three good results out of 12 patients treated though donor cells were not demonstrated in the recipients blood or marrow

Tocantins (personal communication) in a smaller series of similar cases observed a transient increase in the number of reticu locytes and platelets in the patient s blood following injection of homologous marrow but this never persisted for more than three weeks

Surgical Replacement Therapy in Agammaglobulinaemia

As we have seen in Chapter 4 (p 65) a few attempts have been made to treat pa tients with congenital agammaglobulinae mia by free transplantation of lymphoid tissue or by transplantation of the spleen by vascular anastomosis (*see* also p 549) but *without success So far no untoward results* have been reported but there would seem to be serious risk of a graft against host reaction if as is claimed (p 64) patients with agammaglobulinaemia do not react against homografts In the author s opinion therefore *these procedures are unjustified* in our present state of knowledge

Iso and Homo transplantation Following Irradiation or Administration of Marrow Poisons

The first detailed clinical study to be published was that of Thomas Lochte Lu and Ferrebee (1957) and subsequently short accounts of other cases have appeared (An drews 1958 Heller and Yakulis, 1958 Humble and Newton 1958 Kretchmer, 1958 Tocantins 1958b Atkinson Maho ney Schwartz and Hesch 1959)

The information available including one previously unreported case of McWhirter, Newall and Woodruff is summarized in Table III Although in no case can the treatment be described as completely suc cessful the findings are encouraging and point to some important conclusions which should provide a useful guide for the future It will be convenient to consider these under the following headings

1 Hazards of total body irradiation
2 Hazards of the transplantation
3 Optimal dosage of irradiation
4 Optimal dosage of marrow poisons
5 Optimal dosage of homologous cells
6 Possible harmful effect of blood trans fusion after homotransplantation

Hazards of Total Body Irradiation

The deleterious effects to be expected fol lowing high dosage total body irradiation include radiation sickness infection haem orrhage anaemia damage to the gastro intestinal tract and sterility In addition it seems likely that patients who survive long enough may show a high incidence of neoplastic diseases and may develop other remote sequelae which cannot as yet be predicted

Fortunately it appears that if proper pre cautions are taken surprisingly large doses of irradiation can be given without causing radiation sickness The patient treated by McWhirter Newall and Woodruff (unpub lished) received 500 rads in three divided doses over a period of 36 hours but did not vomit and did not appear to be disturbed by the procedure This was attributed partly to the fact that a supervoltage (4 MeV) machine was used and partly to the heavy sedation which the patient received It seems likely that if the radiation had been spread over a longer period a considerably larger dose could have been used without any immediate ill effects

Infection did not present an insoluble problem in any of the cases cited though it is certainly a major hazard while the pa tient s leucocyte count is depressed Isola

TABLE III

PATIENTS TREATED BY IRRADIATION OR CHEMOTHERAPY AND TRANSPLANTATION OF HAEMOPOIETIC TISSUE

Source of Information	Patient					Irradiation or Chemotherapy	Transplant					Result
	Reference No	Sex	Age	Blood Group	Disease		Tissue	Source	Blood Group of Donor	Dose of Nucleated Cells × 10^{-9}	Route	
Thomas *et al.*, 1957	1	M	44	A	Chronic myeloid leukaemia	90r T.B.I. (250 Kv) over 3 days	Homo. Marrow	26 week foetus		0.25	I.V.	Died 24 hours after injection. Autopsy revealed no evidence of emboli
	2	M	63	A	*Multiple* myeloma	*600r T.B.I. (250 Kv)* over 17 days	Homo Marrow	Normal donor. Iliac crest biopsy	O	1.3 spread over 5 injections	I.V.	Possibly transient production of cells by transplant
	3	M	59		Disseminated carcinoma	Nitrogen mustard *0.2 mg./kg. on 3* successive nights	Homo. *Marrow*	Two normal donors. Iliac crest biopsy		0.18 0.42 (13 days between injections)	I.V.	No evidence of donor type cells in blood
	4	F	59		Carcinoma ovary Peritoneal metastases	*Triethylene* melamine 40 mg. over 8 days	*Homo.* Marrow	*Rib removal at* thoracotomy		1.86	I.V.	No evidence of donor type cells in blood
	5	M	59	O C—	Chronic lymphatic leukaemia	450r T.B.I. (250 Kv) over *8 days*	Homo *Marrow*	Cadaver. *Stored 3 weeks* at −80°C	O C+	3.17 spread over 3 injections	I.V.	Donor type cells present for a time in patient's blood. Most numerous 35th to 43rd day. Disappeared after 52nd day
Tocantins, 1958b	6		16		Leukaemia	150r T.B.I.	Homo Marrow			5.4	I.V.	Complete remission of leukaemia. No evidence of recurrence 4 months after treatment
	7-13				Leukaemia 7 cases	Chemotherapy—no details available	Homo Marrow					*Two cases showed evidence of transient pro*duction of cells by transplants (lasting 10-14 days)
Andrews, 1958	14				Acute lymphatic leukaemia	200r T.B.I.	Homo Marrow	4 donors including both parents			I.V.	Remission of leukaemia for 5 weeks, followed by *marked deterioration in patient's condition*
Atkinson, 1958	15				Acute leukaemia	T.B.I. Entrance dose 400r Exit dose 200r	Iso Marrow	Identical twin		2.65	I.V.	Very ill after irradiation. Improved for 2 weeks following cell injection but then relapsed
Kretchmer, 1958	16				Leukaemia	T.B.I. No details	Homo Marrow	Mother				Donor type cells present several months later
Humble and Newton, 1958	17	F	66		*Lymphatic* leukaemia	*Pelvis irradiated to a* skin dose of 2500r	*Homo* Marrow	*4 donors.* Iliac crest biopsy		1.06	I.S.	Donor cells present in host marrow 1 month after injection. Patient alive and much improved 5 months after injection but still had leukaemia
	18	M	67		Aplastic anaemia	*Pelvis and upper* femora irradiated to a skin dose of 2800r. Followed by 15 mg *nitrogen mustard*	*Homo* Marrow	*3 donors.* Iliac crest biopsy		0.90	I.S.	One month after injection blood and marrow counts better than before treatment. Died 2 months after injection from bronchopneumonia.
Heller and Yakulis, 1958	19	M	36	O M CDe/cDe	Hodgkin's disease, and aplastic anaemia induced by triethylene melamine	Triethylene melamine 15 mg. over 10 days, ending about 3 *weeks before marrow* injection. Prednisone.	Homo Marrow	4 donors	(a) N (b) N (c) E (d) E	3.0	I.V.	Blood contained 50-56% N cells and 30-40% E cells 90 days after transplantation. Thereafter these cells became less numerous and by 140 days had completely disappeared. Seven months after the transplantation haemopoiesis appeared to be normal and the Hodgkin's disease was in remission
McWhirter, Newall and Woodruff (unpublished)	20	M			Disseminated seminoma	500r T.B.I. (4 MeV) over 36 hours	Homo Foetal liver	*3 human* foetuses, 12, 14 and 16 weeks' gestation		*5.31* spread over 3 injections (Includes *hepatic cells*)	*I.V.*	*Blood leucocyte and platelet count* fell and 11 days after irradiation was virtually zero. Fifteen days after irradiation leucocyte count had risen to 1400/c.mm. but cells were all mono*nuclear. Patient* died 19 days after irradiation from massive haemorrhage associated with persisting thrombocytopenia.

tion and administration of antibiotics help to minimize the risk

Haemorrhage is a grave danger in heavily irradiated patients and was responsible for the sudden death of the patient treated by McWhirter *et al* One of the main factors responsible is the thrombocytopenia which occurs as a result of the destruction of mega karyocytes but there may also be impaired production of various chemical factors con cerned in blood clotting It should be pos sible to replace missing chemical factors by giving fresh homologous serum but the problem of effectively replacing blood plate lets has not yet been completely solved

Anaemia does not constitute an important danger because it can easily be corrected by blood transfusion As we shall see however this procedure may prove deleterious to the transplanted tissue unless certain precau tions are adopted (p 467)

There has been no evidence of serious damage to the gastro intestinal tract in the reported cases but as larger and larger doses are used this may prove to be a limiting fac tor (*see* e g Cronkite 1958) Sterility seems inevitable unless it becomes possible to re move the gonads during the irradiation and replace them afterwards in such a way that they function satisfactorily This should be feasible in the case of the ovary but seems technically much more difficult in the case of the testis In the light of experiments in rodents it seems possible that renal damage may be a limiting factor but if this proves to be so it should be feasible to remove a kidney before the patient is irradiated and replace it afterwards

Hazards of the Transplantation

It seems clear from the cases recorded that there is no appreciable danger of pulmonary embolism following intravenous injection of large numbers of cells provided that care is taken to eliminate large cell clumps by passing the suspension through an appro priate screen There is therefore no need to use intra arterial or intra aortic injection and indeed the experience of Humble and Newton (1958) shows that this last proce dure is not without danger

The immunological hazard of injecting homologous immunologically competent cells to humans following total body irra diation cannot yet be assessed It may well prove to be serious however and it is conceivable that the patient reported by Humble and Newton as dying of broncho-pneumonia two months after injection of homologous marrow (Case 18 Table III) died from this cause

It seems likely that this could be avoided by using haemopoietic tissue from a foetus at a sufficiently early stage of development There is some evidence that a slight degree of tolerance to homografts can be induced in the human infant at birth (Woodruff 1957b) and it therefore seems a reasonable guess that complete tolerance could be in duced in a foetus of six months gestation If this is so then haemopoietic cells from a foetus of this age or less could probably safely be injected into an irradiated patient In practice foetuses of six months gestation are rarely available On the other hand foetuses of fourteen to sixteen weeks gesta tion can be obtained fairly frequently and were used in the case treated by McWhirter Newall and Woodruff The cell suspensions were prepared from the liver because at this period of gestation haemopoiesis occurs almost exclusively in this organ It remains to be seen whether the cells obtained from such immature foetuses are capable of pro-viding adequate replacement of the whole range of cells necessary for the formation of blood in an adult

Optimal Dose of Irradiation

The optimal dose of irradiation may be defined as the least dose which will abolish the host s immunological competence or alternatively will reduce it sufficiently to allow the transplanted cells to survive and

multiply throughout the life of the host, and in addition, in patients suffering from neoplastic conditions, will completely eradicate the disease.

It seems likely that the optimal dose will vary within wide limits depending *inter alia* on the disease from which the patient is suffering. Conceivably it may sometimes exceed the lethal dose,* in which case the condition obviously cannot be cured by this means. On the other hand it seems clear, as Ferrebee and Thomas (1958) and others have pointed out, that the doses employed hitherto have been much too small, and that the tendency in the immediate future will be to use larger and larger doses.

Optimal Dose of Marrow Poisons

It seems clear that in the few reported cases in which marrow poisons have been used with the object of making an individual receptive of homologous haemopoietic tissue, they have achieved little or nothing. It remains to be seen whether, with any of the substances at present available, it is possible to arrive at a dose which is both effective and safe.

Optimal Dose of Homologous Cells

Thomas, Lochte, Lu and Ferrebee (1957) have suggested that between 4,000 million and 40,000 million nucleated marrow cells might be expected to be an effective dose in man, on the basis that between 1 million and 10 million nucleated cells is an effective dose in a mouse weighing 30 g. and between 200 million and 2,000 million nucleated cells is an effective dose in a monkey weighing 3 kg. They therefore find it reassuring to recall that between 1,000 million and 2,000 million nucleated marrow cells may be obtained from a large foetus, an adult rib, or a biopsy of the ilium, and about 50,000 million from an adult cadaver.

In the absence of precise information it seems reasonable to use this estimate of the required dose as a guide, but it is important to realize that it may be very inaccurate. The dose suggested for a mouse is probably on the small side, and extrapolation from one species to another on the basis of body weight may well be erroneous. Moreover the nucleated cell count may be very misleading unless the technique by which the cells were obtained is specified. Thus material obtained by aspiration biopsy of the ilium, as Tocantins (1958a) has pointed out, contains much blood in comparison with that obtained, for example, from an excised rib.

If foetal tissue (and in particular foetal liver) is used, the dose required may be quite different. It seems highly probable that the hepatic parenchymal cells are of no value, while the haemopoietic cells may be more effective or less effective than the same number of adult nucleated marrow cells. In the case (No. 20) cited in Table III, the patient received a total of 5,300 million nucleated cells, inclusive of hepatic parenchymal cells, and this dose was probably considerably too small.

Possible Harmful Effect of Blood Transfusion

In the case treated by McWhirter *et al.* the patient received several transfusions of fresh blood. This may have been responsible, or partly responsible, for the sudden disappearance of donor cells from the circulation, for it is conceivable that, even if the host's immunological competence were completely abolished, the transplanted haemopoietic cells may have been destroyed in consequence of an immunological reaction mediated by leucocytes (foreign to both the haemopoietic tissue donor and the host) introduced in the transfusions of fresh blood. The matter is at present under investigation but meanwhile it would seem wise to use only stored blood which normally contains few, if any, viable leucocytes.

*In this context the term lethal dose denotes the minimum dose which will kill the patient in spite of successful repopulation with haemopoietic cells

Transplantation of Other Tissues After Irradiation and Replacement of Haemopoietic Tissue

Merrill and his colleagues (cited by Ferrebee and Thomas 1958) irradiated a woman whose sole functioning kidney had been removed and gave her a transplant of bone marrow from her brother Subsequently she was given a kidney transplant from a hydrocephalic child The procedure was unsuccessful but this is scarcely surprising in view of the failure so far to establish homologous bone marrow in human patients Moreover, even if the marrow graft had been successful the chance of the kidney surviving permanently would seem to be small in view of the fact that the kidney and the marrow came from different adult donors

A second patient treated by Merrill *et al* —a boy whose sole functioning kidney had been severely damaged—was irradiated and received bone marrow from his mother and also foetal liver, but subsequently his condition was so poor that a kidney graft was not attempted

STORAGE OF HAEMOPOIETIC TISSUE

Billen (1957) reported that lethally irradiated mice which received an injection of homologous marrow cells which had been cultured *in vitro* for 24 days survived despite the fact that many of the cells had lost their specific morphological characteristics and resembled plasma cells If this finding is confirmed it is conceivable that marrow cells cultured *in vitro* may one day be used clinically provided that the danger of neoplastic mutations can somehow be overcome

At present however the method of storage which appears to offer most promise of success is to freeze the marrow after treatment with glycerol and store it at about −79°C Barnes and Loutit (1955a, b) succeeded in preserving infant mouse spleen in a viable state for as long as 83 days in this way, and subsequently Schwartz Repplinger, Congdon and Tocantins (1957) showed that glycerol treated isologous adult mouse bone marrow stored at −70°C for 8 days was as effective as fresh isologous marrow in promoting the recovery of mice irradiated to 900r In these last experiments femoral marrow was obtained by irrigating the marrow cavity with Tyrode s solution containing glycerol (15 per cent) and Sequestrene,* and sealed in glass ampoules The ampoules were placed in the freezing compartment of a Barnes Loutit apparatus and the temperature was lowered at a rate of 1°C /minute to −15°C and thereafter at not more than 10°C /minute to −70°C *The ampoules were stored at −70°C and* thawed in a water bath at 37°C when required

Human marrow has also been stored frozen by a number of investigators

In one of the cases reported by Thomas *et al* (1957) glycerol treated marrow which had been stored frozen for 3 weeks was used and donor type cells were subsequently demonstrated for a time in the patient s blood

In the two cases reported by Kurnick Montano, Gerdes and Feder (1958) in which marrow autografts were used the cells were suspended in Tyrode s solution containing glycerol (30 per cent) and heparin (5 units/ml marrow) sealed in glass ampoules frozen in a Barnes Loutit apparatus, and stored in a dry ice cabinet The suspension was thawed rapidly by immersing the ampoules in a water bath at 37°C and trans

*Disodium ethylenediamine tetra acetic acid It seems illogical to use an electrolyte solution containing Ca^{++} and Mg^{++} in conjunction with Sequestrine and Repplinger (personal communication) now uses an isotonic saline which contains no salts of either Ca or Mg

ferred to a plastic bag. At this stage the cells were markedly hypertonic owing to their high glycerol content and it seemed likely that they would be lysed if infused directly. To avoid this danger the procedure devised by Sloviter (1956) for preparing thawed erythrocyte glycerol mixtures was adopted, i.e., glucose (which diffuses very slowly through the human red cell membrane in comparison with glycerol) was added, and the suspension was subsequently diluted with an isotonic electrolyte solution.* This caused some clumping and the cells were therefore separated and re-suspended in 5 per cent glucose saline. As we have seen (p. 463), it was estimated that 75-80 per cent of the cells obtained from the donor remained viable. The validity of the criterion used is certainly open to question, but there seems no doubt from the bone marrow studies in the first recipient that sufficient cells remained viable for the procedure to be clinically effective.

*Sloviter used isotonic saline whereas Kurnick *et al.* used Tyrode's solution.

According to Ferrebee (1958a, b) the cell suspension should be frozen slowly (1°C. *per minute*). He advises suspending the cells in 15 per cent glycerol (but is not certain that this is really necessary), while Berman (1958) advocates 20 per cent glycerol. Humble and his colleagues (*see* Pegg and Trotman, 1959) use 15 per cent glycerol, and cool at the rate of 1°C./minute to —15°C. and thereafter at not less than 5°C./minute. The author (unpublished) uses a similar procedure, but rather less elaborate cooling equipment.

THE OUTLOOK FOR TRANSPLANTATION OF HAEMOPOIETIC TISSUE IN CLINICAL PRACTICE

Autotransplants

The notion of removing some bone marrow before carrying out extensive radiotherapy and replacing it afterwards appears logical, and it is surprising that it has not been more widely used.

In the author's view this procedure is at present justifiable if, and only if, the following four criteria are fulfilled:

1. The patient is suffering from some form of neoplastic disease.
2. It seems likely that he could be cured, or given worthwhile palliation, by radiotherapy in a dosage which would produce dangerous bone marrow aplasia but would not otherwise be lethal.
3. Less hazardous forms of treatment have either failed or offer little or no prospect of success.
4. There are no obvious skeletal metastases

The procedure seems likely to find its greatest application in the treatment of pulmonary metastases, especially when they are not very large and the primary tumour was reasonably radiosensitive.

It would seem best, if the patient's thorax has not previously been irradiated and the operative risk appears to be reasonable, to resect one or more ribs subperiosteally, or alternatively, if the pelvis has not been irradiated, to remove marrow from the ilium by open operation, and to prepare a cell suspension in a balanced saline solution with 10 per cent compatible homologous serum, for intravenous injection. It may prove desirable to increase the proportion of lymphocytes in the transplant and this could be done with little risk of including neoplastic cells by removing the patient's spleen and preparing a cell suspension from it which could be added to the marrow suspension or injected separately. As the cells are autologous there should be no risk of a graft

against host reaction If the patient is not considered fit enough for splenectomy lymph nodes could be used instead and if these were selected judiciously the risk of including neoplastic cells should not be great

If the risk of open operation appears prohibitive iliac crest biopsy with a Bierman needle (Bierman and Kelly 1956) seems to be a possible alternative although it may be difficult to obtain enough cells

The method of storage will depend on the duration of the radiotherapy For short periods it would seem best to keep ribs or iliac bone intact at about 4°C and prepare the suspension just before it is required whereas for long periods a suspension should be made in a medium containing glycerol frozen in two stages as already described and stored at −79°C or lower At the moment however it is impossible to say what constitutes a short period or whether these methods will prove to be the best in the long run

There would seem to be little danger of the graft failing to provide adequate replacement so long as the patient receives an adequate number of viable cells but the hazards of irradiation may be considerable The nature of these hazards and the means which can be used to minimize them have already been considered (p 461) The operative risk is by no means negligible and is still greater if splenectomy is performed in addition to removing a rib or a piece of ilium but will probably only occasionally appear prohibitive

Removal and replacement of bone marrow is being used in a similar way in conjunction with the treatment of neoplastic diseases by chemotherapy (Westbury Humble Newton Skinner and Pegg 1959 Woodruff unpublished)

Another use for autotransplants will arise if long term storage does prove feasible for it would then seem reasonable to obtain marrow (if necessary by open operation) from people who are exposed to the hazard of acute radiation injury and store it in case the person concerned is involved in an accident and requires marrow replacement

Isotransplants

If an identical twin is available and willing to act as donor, isotransplantation of bone marrow, and in some cases also lymphoid tissue would seem to be indicated in the following conditions

1 Aplastic anaemia
2 Acute radiation injuries
3 Leukaemia
4 Extensive neoplastic disease other than leukaemia

In leukaemia and other neoplastic conditions irradiation or chemotherapy will precede the transplantation In leukaemic patients it would seem to be essential if irradiation is used to irradiate the whole body simultaneously and much existing equipment will have to be modified before it can be used for this purpose At present so-called supervoltage therapy machines operating at 2 1 MeV provide the best available source of total body irradiation but machines operating at 100 or more MeV may come into use and may—or may not—prove advantageous for this purpose

Homotransplants

If homotransplantation of bo
were as effective as and no more
than auto or iso transplantation
be indicated in the conditions l
preceding section and also as a
to or in association with
of a kidney or other organ from
donor

The premise on which this
based is at the present time not
in the author s opinion—and this
speculation—methods will be
making permanent human cl
pears likely that this will first be

by irradiation, or by some combination of irradiation, radiomimetic drugs and chemical protective agents, but that these will be replaced later by procedures which are biologically less crude.

Since prolonged survival of homologous haemopoietic tissue has not yet been achieved in man the risk of severe secondary disease cannot be assessed. In conditions such as leukaemia, secondary disease, if not too severe, might actually be advantageous in helping to eliminate any viable neoplastic cells remaining after the irradiation. In any event it seems likely from experiments in animals that methods of replacing erythropoietic and granulopoietic tissue without causing serious secondary disease will be devised. It may be that in man bone marrow alone will prove safe, and if not it may possibly be made safe by administering chemical agents toxic to lymphocytes; alternatively transplants of foetal liver may be used although, as we have seen, other problems then arise.

There would still remain the problem of replacing immunologically competent tissue in sufficient quantity to ensure that the patient was not an immunological cripple without at the same time causing a fatal graft against host reaction. The observation that animals may recover from quite severe secondary disease and remain stable chimeras thereafter—though at present inexplicable—gives some ground for believing that this problem too will be solved.

CHAPTER 23

Transplantation of Endocrine Tissues (Excluding Gonads)

INTRODUCTION

Transplants of endocrine tissues are of special interest from the biological point of view, partly because they can be subjected to specific hormonal stimulation and partly because a wide variety of tests of functional activity can be applied to them

They are at the moment of less interest to surgeons because there are but few indications for autotransplantation, and homotransplants cannot be relied on to provide adequate replacement Moreover, substitution therapy, in the form of administration of the appropriate hormone, is remarkably effective in many endocrine deficiencies Nevertheless, if and when the biological homograft problem is solved, transplantation of endocrines will almost certainly come to be widely used in clinical practice because a living transplant should prove much more effective than the best substitution therapy in maintaining homeostasis

TRANSPLANTATION OF PITUITARY

HISTORY

Animal Experiments

Autotransplants and Homotransplants

The early experiments with autotransplants and homotransplants of pituitary in rodents (Sacerdotti 1903 1905 Carraro, 1909a, b, Clairmont and Ehrlich, 1909, von Saar 1909) and dogs (Del Conte, 1907, Clairmont and Ehrlich 1909 Cushing, 1909, Crowe, Cushing and Homans 1909) were unsuccessful, the transplants being rapidly destroyed, though some dogs which received transplants following hypophysectomy appeared to survive a little longer than hypophysectomized controls

It was shown subsequently, however, that the tissue of the anterior lobe may survive for long periods after autotransplantation, and under certain conditions after homotransplantation The tissue of the posterior lobe does not survive permanently, but if, as often happens, it is replaced by fibrous tissue, the end point of survival is rather indefinite In many of the reported experiments the transplants appear to have been endocrinologically quite inactive, but in others evidence of functional activity of one kind or another is presented We shall examine shortly the reasons for these apparently conflicting findings and the conditions which determine the functional activity of viable anterior pituitary transplants, but first we shall review some of the experiments in which transplants survived but apparently did not function

Hohlweg and Junkmann (1932) studied intrarenal pituitary transplants in rats Both male and female hosts were used, and they

were castrated at the time of transplantation. Four weeks later the animals were killed and it was found that whereas the host's own pituitary showed the histological changes which typically occur after castration the transplants failed to do so. This observation was subsequently confirmed by Desclin and Grégoire (1936) and Westman and Jacobsohn (1940). To explain their findings Hohlweg and Junkmann postulated that there was a cerebral sexual centre through which the gonads exercise their action on the hypophysis; the true explanation of the phenomenon did not become apparent however until the discovery of the pituitary portal circulation.

Iwao and Ochiai (1935) and Ochiai (1939), working with rabbits, studied the behaviour of pituitary transplants in the bone marrow and the greater omentum respectively. In their papers they speak of homotransplants, though it appears from the description they give that they did in fact use autotransplants. The transplants became vascularized and, except for some central necrosis in the larger transplants, showed no histological abnormality, and in particular no lymphocytic infiltration.

Buxton (1936) investigated the behaviour of pituitary transplants in the anterior chamber of the eye in rats. Six out of 35 autotransplants, and one out of seven *homotransplants*, became vascularized and were found on histological examination three to six weeks later to contain recognizable anterior lobe tissue, but no posterior lobe tissue. Only three of the recipients were hypophysectomized, and it was in one of these animals that the successful transplant was obtained. Richter and Eckert (1937b), who also used rats, were more successful, and reported long survival of anterior lobe tissue in the anterior chamber of the eye in 13 out of 14 hypophysectomized recipients; and Haterius, Schweizer and Charipper (1935) reported that anterior lobe tissue persisted for months in pituitary homotransplants in the anterior chambers of intact guinea pigs.

Gardner and Hill (1935) reported survival of intratesticular pituitary homotransplants in mice.

Phelps, Ellison and Burch (1939) made intramuscular pituitary homotransplants from mature male or female rats to hypophysectomized females of the same inbred strain. The transplants were left in place for 5-60 days. Many, including a few 60-day transplants, contained intermediate and posterior lobe, as well as anterior lobe, tissue. Some of the recipients were given injections of oestrogen, and in these the anterior lobe tissue was particularly abundant and showed histological changes of the kind which occur in the normal pituitary following oestrogen administration (i.e. a decrease in the number and granularity of the chromophil cells, and an increase in the number of chromophobe cells).

Evidence of functional activity may take many forms, including maintenance of growth, and gonadotrophic, mammatrophic, adrenotrophic and thyrotrophic activity.

May (1935) reported that two young rats, which had stopped growing as a result of hypophysectomy performed two months previously, began to grow following homotransplantation of anterior pituitary from new-born rats to the anterior chamber of the eye. This observation was confirmed and extended by Martinovitch (1949, 1950a), who observed striking growth in hypophysectomized rats following homotransplantation of either fresh pituitary, or pituitary which had been cultured for 32 days *in vitro*, from infantile donors.

Maintenance of the histological structure of the testis, and in some cases restoration of fertility, has been observed following pituitary transplantation in hypophysectomized animals by a number of observers. The transplants were placed in various sites

including the anterior chamber of the eye (May, 1935 Schweizer, Charipper and Kleinberg, 1940, Cutuly, 1941), the testis (Hill and Gardner, 1936), the axilla or groin (Haymaker and Anderson, 1936), and the empty sella turcica (Greep, 1936).

Reports concerning the maintenance of the female genital tract are far from consistent May (1937) found that normal oestrous cycles were maintained in hypophysectomized adult female rats which received pituitary homotransplants from new born donors to the anterior chamber of the eye, but the completeness of the hypophysectomy has been called in question because the cycle continued in one animal after removal of the eye containing the transplant, and several other observers who have studied the effect of transplants in the anterior chamber have not confirmed May's findings Thus for example Martins (1936a, b) who also used rats, found that three quarters of his animals showed no oestrous cycles and that in the remainder the cycles were irregular while Schweizer, Charipper and Haterius (1937), who used guinea pigs, found that about two months after transplantation the recipients went into a state of continuous oestrus On the other hand Greep (1936) found that hypophysectomized rats, which had received homotransplants of pituitary from either male or female donors to the sella, not only showed regular oestrous cycles, but were capable of becoming pregnant, of giving birth to live young, and of lactating Further evidence supporting the view that pituitary transplants may exert a gonadotrophic effect was provided by Silberberg Silberberg and Leidler (1951) who observed that subcutaneous intrastrain anterior pituitary homotransplants promoted proliferation of follicular and lutein tissue in intrasplenic ovarian autografts in mice

Hypertrophy of the mammary glands was observed in guinea pigs following hypophysectomy and homotransplantation of pituitary to the anterior chamber by Schweizer, Charipper and Haterius (1937). Still more striking results were obtained by Loeb and his associates (Loeb and Kirtz, 1939, Loeb, Blumenthal and Kirtz, 1944, Silberberg and Silberberg, 1950), who observed in mice that the stimulating effect of pituitary transplants might reach such proportions as to precipitate the development of mammary carcinomata, it appeared, however, that the effect was not due to mammatrophin secreted by the transplant but was mediated by the ovary, because carcinomata did not develop in ovariectomized animals

Generally speaking the effect of hypophysectomy on the structure of the adrenal cortex has not been greatly modified by pituitary transplants Schweizer and his colleagues (Schweizer, Charipper and Haterius, 1937) reported at first that the adrenals were well maintained in guinea pigs following hypophysectomy and homo transplantation of pituitary to the anterior chamber, but subsequently they stated that the transplants had little effect, irrespective of whether the hypophysectomy was performed at the same time as transplantation (Schweizer, Charipper and Kleinberg, 1940) or one to three weeks later when the transplant was well established (Schweizer and Long, 1950a) Cutuly (1941), Westman and Jacobsohn (1940), and Cheng, Sayers, Goodman and Swinyard (1949), found that anterior chamber transplants in rats were no more effective, whereas according to Cutuly (1941) transplants in the sella exerted a marked effect According to McDermott, Fry, Brobeck and Long (1950), however, even transplants in the anterior chamber may secrete ACTH in response to subcutaneous or intra ocular injection of adrenaline; and Furth, Gadsen and Upton (1953) have obtained convincing evidence of ACTH secretion by two transplantable pituitary tumours which developed in mice exposed to ionizing radiation

The capacity of pituitary transplants in

the anterior chamber to maintain the structure and function of the thyroid gland following hypophysectomy also appears to be very limited, although the published observations are not entirely consistent. Martins (1936b) and Cutuly (1941) observed virtually no effect. Schweizer, Charipper and Haterius (1937) reported that the thyroid was well maintained, but subsequently Schweizer and Long (1950b) found that only three out of six hypophysectomized guinea pigs bearing anterior chamber pituitary transplants gave evidence of thyrotrophic activity as judged by acinar cell height, and even in these animals the activity was much below normal. On the other hand Greer, Scow and Grobstein (1953) found in mice that, although the weight of the thyroid was not maintained following hypophysectomy and transplantation of pituitary to the anterior chamber, the capacity of the thyroid to take up radioactive iodine was maintained at about two-thirds of its normal level.

The situation was clarified by the discovery of the role of the hypothalamic-pituitary portal circulation in controlling the secretion of the anterior pituitary (*see* Harris, 1948, for review), and by the observations of Harris and Jacobsohn (1952) on the functional activity of pituitary transplants in different sites.

In their first experiment Harris and Jacobsohn transplanted pituitary from 2 to 10 day old male and female rats of an inbred strain to their mothers. Transplants placed under the median eminence of the tuber cinereum acquired vascular connexions with the primary plexus of the hypophyseal portal vessels, remained viable for long periods, and attained normal function as judged by the following criteria: (a) occurrence of regular oestrous cycles, pregnancy and lactation; (b) maintenance of the weight and normal histological structure of the ovaries and adrenals, and (c) maintenance of the normal histological structure of the thyroid. Transplants placed beneath the temporal lobe of the brain became well vascularized, but not from the pituitary portal vessels, and although they remained viable for a long time they showed little or no evidence of function. Transplants placed in the emptied pituitary capsule did not unite quite as well as transplants in the other two situations; moreover, even when they became vascularized they did not acquire connexions with the portal system and they showed little or no evidence of function.

In their second experiment Harris and Jacobsohn studied homotransplants of pituitary from adult male and female rats to adult females. Both intrastrain and interstrain transplants were used, but the reason for this is not apparent, and it is not always clear from the description given which type of transplant is being referred to. Again, however, transplants placed beneath the median eminence of the tuber cinereum became vascularized from the pituitary portal system, and fulfilled the criteria of functional activity described previously despite the fact that when removed and sectioned six weeks after transplantation they often showed marked lymphocytic infiltration. Transplants placed below the temporal lobe often became vascularized, but not from the portal circulation. They showed no evidence of function, and the genital tract, adrenals, and thyroid of the host became atrophic.

Finally, Harris and Jacobsohn transplanted pituitary from adult male rats to other adult males of the same or a different strain. Transplants placed beneath the median eminence of the tuber cinereum became vascularized as in the other experiments from the portal circulation, but they showed marked lymphocytic infiltration. The testes were partially maintained in two out of four hosts, and showed spermatogenesis. The seminal vesicles were maintained in one animal, and the adrenal glands were

partly maintained in all animals Transplants placed below the temporal lobe were well vascularized but not from the portal vessels and the testes seminal vesicles and adrenals of the host all became atrophic

Harris and Jacobsohn concluded from these experiments that the secretion of anterior pituitary hormones is under hypothalamic control and is mediated by the pituitary portal vessels This hypothesis is now generally accepted and there is no doubt that when anterior pituitary tissue is isolated from the portal circulation its function is to say the least grossly impaired In the light of all the experimental data we have been considering however it would not seem justified to conclude that in a free heterotopic transplant the production of all the anterior pituitary hormones is totally abolished

In addition to experiments of the kind we have been discussing there have been many others which we shall mention only in passing in which homotransplants were used merely as depots of pituitary hormones (*see* e g Smith 1926 1927 1930 Watrin and Florentin 1932 Gorbman 1950) and in which the period of observation was very short or alternatively the transplantation was repeated every day or two

Heterotransplants

It has been shown by various investigators that pituitary heterotransplants may act as hormone depots (Emanuel 1931 Loeb 1932a b 1933a b Saxton and Loeb 1937 Silberberg and Silberberg 1938)

According to Kylin (1937a) intraperitoneal heterotransplants of calf pituitary in rabbits undergo central necrosis but subsequently regenerate from surviving tissue at the periphery and they bring about a rise in blood pressure and in the blood sugar level both of which persist for months (Kylin and Koranyi 1938) Westman and Jacobsohn (1942) on the other hand found that pituitary heterotransplants in rats soon ceased to be functionally effective and were absorbed and this is clearly more in accordance with what is known of the behaviour of heterotransplants of other tissues

Clinical Observations

Homotransplants

Pituitary homotransplants have been used in a number of patients suffering from emaciation sometimes associated with amenorrhoea Vogt (1935) who reported one such case used an intramuscular transplant from a new born infant Kubanyi (1941a b 1949) cut up his transplants into small pieces and buried these among sympathetic nerve fibres surrounding the carotid artery Some very successful results have been reported but it seems likely that many of the patients were suffering from anorexia nervosa and that the observed improvement was psychogenic in origin

Bogoraz (1938) made homotransplants of pituitary by vascular anastomosis to 11 dwarfs The glands were obtained from young subjects who had been killed in accidents and each was removed complete with its vascular connexions including short segments of both internal carotid arteries One internal carotid was then anastomosed to the patient s brachial artery and if this anastomosis appeared satisfactory the other segment of internal carotid was discarded Four of the patients had been followed for more than 18 months one of them had grown 15 6 cm in height and had ceased to look like a dwarf while the others had grown to a lesser extent Growth ranging from 2 to 6 cm was observed in several other patients whose height had been stationary for some considerable time prior to the operation Increase in the size of the testes and in sexual activity occurred in some of the male patients and menstruation was re established in several females

Hirsch (1937) treated one patient suffering from diabetes insipidus with a homo

transplant of posterior pituitary, obtained from a cadaver, to the rectus sheath. The patient's daily output of urine dropped in the course of 10 weeks from 7,500 ml. to between 6,000 and 2,000 ml., while the maximum specific gravity of the urine increased from 1005 to 1019. The improvement persisted, and two and a half years later the daily output of urine was usually between 1,300 and 1,500 ml., occasionally rising as high as 3,600 ml., while the specific gravity of the urine ranged from 1005 to 1015.

Heterotransplants

Favourable results have been reported in patients with various endocrine and other disorders including amenorrhoea, anorexia nervosa, Simmond's disease, panhypopituitarism, dystrophia adiposo-genitalis, and dwarfism, following heterotransplantation of whole pituitary, or of the anterior lobe of the pituitary, from calves, goats, sheep, pigs, and occasionally monkeys (Ehrhardt and Wiesbader, 1928; Bergmann, 1934; Kylin, 1936, 1937b, 1938, 1939a, 1940a, b, 1941; Kylin and Kjellin, 1936; Ehrhardt and Kittel, 1937; Sjovall, 1938; Luchetti, 1939; Pontoppidan, 1941; Jerlov, 1946; Paufique and Guinet, 1948; Damm, 1949; Westman, 1949; Damm and Horst-Meyer, 1950; Fellinger, 1950; Horst-Meyer, 1950; Hammerschmidt and Korting, 1951; Pahlsson, 1951; Borell, Diczfalusy and Westman, 1952; Wassmann, 1952). The transplants were placed in various sites including the subcutaneous tissue, the rectus sheath and the omentum. Ehrhardt and Wiesbader (1928) reported that the transplants were usually absorbed within two to five months, but clinical improvement, according to Ehrhardt and Kittel (1937), usually lasted for about a year. Even more prolonged improvement, and in some cases permanent cure, has been reported, notably by Kylin and his associates.

It seems likely that some at least of the good results were psychogenic in origin, and that the remainder were due, as Westman (1949) and others have suggested, to absorption of hormones from a transplant in process of destruction. This suggestion is supported by Westman's observation that in 35 per cent of his cases suppuration occurred in the vicinity of the transplants but that this did not appear to affect the result. It is, however, surprising that such long continued improvement occurred in some patients showing convincing evidence of the presence of an organic disorder of the pituitary.

Good results following pituitary heterotransplants have also been reported in a miscellaneous group of disorders including psoriasis (Kylin, 1939c), alopecia areata (Damm, 1949), undescended testis (Lombard and Gros, 1939), rheumatoid arthritis (Edström and Westman, 1942; Edström, 1950; Edström and Thune, 1951), and pain associated with inoperable cancer (Gumrich, 1951).

Heterotransplants of whole pituitary or of the posterior lobe of the pituitary have been used in patients with diabetes insipidus by Sacorrafos (1933), Houssay (1937), Matolay (1938), Kylin (1939b), Barath (1939), Rissel (1940), Azérad (1946), Keibl (1948), Schertenleib (1948), Hornbostel (1949), Emmrich and Horst-Meyer (1951), and others, and in patients with disturbances of carbohydrate metabolism associated with lesions of the hypothalamus by Horst-Meyer (1951). Many of the patients have shown remarkable improvement, but as a rule this has not lasted for more than a couple of weeks, although improvement of much longer duration has been recorded by some observers including Matolay, Kylin, Barath, Keibl and Hornbostel. It seems almost certain that the transplants served only as depots of pitressin, although once again it is difficult to account for the prolonged improvement which occurred in some of the patients.

Transplantation of Pituitary in Present Day Surgery

In the author's opinion there are at present no indications for using either homotransplants or heterotransplants of pituitary in clinical practice The beneficial results which have been claimed for these procedures can all be reproduced, and indeed can be improved upon, by adequate hormone replacement therapy Moreover, even if the immunological homograft problem is solved, pituitary homotransplants are unlikely to be functionally fully effective unless they are placed within the cranial cavity in such a position that they can establish vascular connexions with the pituitary portal circulation, and such an operation is unlikely to become popular unless it could be shown to give decidedly better results than simpler forms of therapy

TRANSPLANTATION OF ADRENAL

HISTORY

Animal Experiments

Early Work

Canalis (1887) appears to have been the first to transplant adrenal tissue In the course of a study of traumatic lesions of the adrenal in rabbits he removed fragments of the gland and buried them in the substances of the kidney, and in one instance cortical cells were demonstrable in the graft when the animal was killed 15 days later

During the next 15 years adrenal transplantation was attempted by various other investigators in frogs (Abelous 1892 Gourfein, 1896 Langlois 1897), rats (Boinet, 1895, Poll, 1898, 1899, Strehl and Weiss, 1901) rabbits (Hultgren and Andersson, 1899 Schmieden, 1902, 1903) and cats (Hultgren and Anderson 1899) The transplants for the most part were autologous, and they were placed in various sites including the subcutaneous tissue, the muscles, the peritoneal cavity, the kidney and the liver, but with a few exceptions the recipients succumbed following bilateral adrenalectomy in about the same time as non grafted controls of the same species In many instances the transplants were completely absorbed, but Poll (1898, 1899) made the important observation that cortical tissue sometimes survived whereas the medulla invariably disappeared and was replaced by connective tissue

Cristiani and Cristiani in 1902 published an important paper recording their experiences with intraperitoneal adrenal autografts in rats They found that an intact gland dropped into the peritoneal cavity usually became attached to the omentum The central part of the gland underwent degeneration and necrosis subsequently the cortex regenerated and developed its three characteristic zones, but the medulla did not regenerate With grafts in the form of multiple small pieces of adrenal regeneration of cortical tissue was even more rapid, and some medullary tissue occasionally persisted With very few exceptions, however, removal of all remaining adrenal tissue apart from the graft proved rapidly fatal, and Cristiani and Cristiani concluded that the adrenal medulla was essential for life It is now known however that this is not the case

Busch and his colleagues (Busch and van Bergen, 1906, Busch, Leonard and Wright 1908) obtained evidence of function with two intrarenal transplants in rabbits, one autologous and the other homologous The recipients were kept under observation for 77 days and 36 days respectively, during which time they remained in good health The kidney bearing the transplant was then removed, and in each case death occurred within three days

Stoerk and von Haberer (1908) made autologous pedicle transplants of the adrenals to the kidneys in rabbits, dogs and cats, and observed that in some instances medullary tissue survived as well as cortical tissue.

Further experiments with free transplants were reported by Coenen (1906), Shiota (1909) and others, but these added little to what was already known. Crowe and Wislocki (1914) were the first to study free transplants of adrenal tissue in dogs, but only a small amount of cortical tissue survived and it did not appear to be functionally effective. Interest in adrenal transplantation lapsed, but revived with the work of Jaffe, Wyman, Ingle and others between the wars.

Further Experiments with Autotransplants

More recent experiments with free autotransplants of either the whole or a large part of the adrenal gland have confirmed that the central part of the transplant becomes necrotic, but under suitable conditions the cortex regenerates from the capsule and the zona glomerulosa (Ingle and Higgins, 1937, 1938a; Williams, 1945, 1947). The medulla does not regenerate. Many sites have been used successfully including skeletal muscle (Jaffe and Plavska, 1926; Dornfeld, 1937; Wyman and Tum Suden, 1937a, b, 1941), the ovary (Pencharz, Olmsted and Giragossintz, 1930; Pencharz and Olmsted, 1931; Martin, 1932; Ingle and Higgins, 1938a; Eversole, Edelmann and Gaunt, 1940), the kidney (Eversole *et al.*, 1940) and the spleen (*v. infra*). Subcutaneous transplants to the pinna (Kroc, 1942) and elsewhere may also regenerate, but have on the whole proved less successful. Most of this work has been done with rats, but some experiments have been performed with guinea pigs (Jaffe, 1927) and other animals. In the guinea pig, as Elliot and Tuckett (1906) first pointed out, a severe inflammatory reaction occurs if medullary tissue is included with the transplant. Jaffe (1927) showed that this is due to the action of adrenaline on guinea pig muscle, and does not occur with transplants of cortical tissue alone. In dogs, Blodinger, Klebanoff and Laurens (1926) found that some cortical tissue survived in free autotransplants to kidney, spleen, omentum or skeletal muscle, but the level of functional activity was never sufficient to maintain life after removal of all other adrenal tissue. Johnson and Johnson (1931) were equally unsuccessful in their attempt to obtain functioning free adrenal grafts in this species.

With transplants by vascular anastomosis (Nilson and Ingle, 1936; Levy and Blalock, 1939) and pedicle transplants (Keeley, Dunphy, Quigley and Bell, 1940) the medulla as well as the cortex may survive.

Adrenal autotransplants fail to regenerate in hypophysectomized animals (Wyman and Tum Suden, 1937a), in animals which retain a large amount of functioning cortical tissue (Wyman and Tum Suden, 1932, 1937a), and in animals treated with active cortical extracts or cortisone unless ACTH is administered as well (Woodruff and Boswell, 1953).

Adrenal autotransplants with venous drainage to the systemic circulation are capable of maintaining life in adrenalectomized hosts under normal conditions (Jaffe, 1926, 1927; Jaffe and Plavska, 1926), and protecting them against a variety of abnormal stresses, for example injection of histamine in a dosage sufficient to cause immediate death in adrenalectomized hosts not bearing grafts (Wyman and Tum Suden, 1937b).

Somewhat surprisingly, adrenal autotransplants to the spleen or mesentery not only regenerate but also appear to be functionally effective, as shown by the fact that they are capable of maintaining life and growth (Butcher, 1948; Bernstein, 1950a; Read, 1951; Rather, 1952), and also sexual potency (Bernstein, 1950b), in animals from which all other adrenal tissue has been re-

moved. Clearly the main venous drainage from these transplants is via the portal system, and in several of the experiments reported the absence of adhesions between the organ bearing the transplant and the parietes makes it appear likely that the whole venous return was by this route. This has been taken as evidence that some at least of the adrenal steroid necessary to maintain life is not inactivated by passage through the liver.

Adrenal autotransplants in appropriate sites, for example the seminal vesicle of the castrated male or the uterus of the spayed female, may produce local androgenic (Katsh, Gordon and Charipper, 1948) or oestrogenic (Weinstein, Schiller and Charipper, 1950) effects.

Further Experiments with Homotransplants

Adrenal homotransplants exchanged between rats of a closely inbred strain behave, as expected, like autotransplants (Ingle, Higgins and Nilson, 1938). With non-inbred animals homotransplants sometimes behave like autotransplants when the donor and host are siblings or, less frequently, when they are cousins (Ingle and Higgins, 1938b) and homografts to the ovary have occasionally been found to survive and function for long periods even when the donor and host were unrelated (Woodruff and Boswell, 1953; Woodruff and Sparrow, 1958).

Homotransplants from foetuses, new-born, and prepubertal rats have been studied by various observers.

Parodi (1904) observed that in rabbits homografts of foetal adrenal to the kidney, liver or sciatic nerve, increased in size, and that this increase was due almost exclusively to proliferation of cortical cells. Sooner or later however degenerative changes occurred, and the transplant was eventually destroyed and replaced by host connective tissue. He made the further interesting observation that the grafts survived longer in young hosts than in old hosts. Neuhauser (1909) also observed survival and proliferation of cortical cells in homotransplants of foetal adrenal to the kidneys of adult rabbits.

Rutishauser and Guye (1936) found that adrenals from new-born rats, transplanted to the kidneys of adults from which one adrenal had been removed, regenerated and functioned. Higgins and Ingle (1938) were equally successful with similar grafts placed in the groin alongside the femoral vessels, whereas Woodruff and Boswell (1953), who used the same technique, found that all the grafts were completely destroyed within four weeks. Jones (1954) obtained results intermediate in character. He transplanted adrenals from new born rats to either the groin or the erector spinae muscle of adrenalectomized adults and found that in 22 out of 86 recipients they survived throughout the period of observation (up to 11 months).

Turner (1939) found that homografts from prepubertal rats regenerated well in the anterior chamber of the eye, and Pomerat, Breckinridge and Gordon (1944) found that grafts of adrenals from new born rats to the brains of adult hosts showed complete differentiation of the cortex, and provided adequate functional replacement for at least 274 days, despite the fact that they became vascularized. Unfortunately, however, the degree of genetic diversity of the animals used by Pomerat *et al.* was not specified.

Homografts of guinea pig and rabbit adrenal which had been cultured *in vitro* were studied by Lux, Higgins and Mann (1937, 1938), and their findings have been discussed in Chapter 6.

Prolonged survival of adrenal homotransplants, associated with functional activity, in hosts previously rendered tolerant by injection of cells from the prospective donor before the development of immunological maturity, has been demonstrated by Wood

ruff and Sparrow (1958) and Medawar and Russell (1958).

Clinical Observations

Homotransplants of normal and hypertrophic adult adrenal and foetal adrenal, and heterotransplants, have been tried many times in patients with Addison's disease. The findings are of considerable biological interest but, in the author's view, little clinical importance.

Homotransplants

Hurst, Tanner and Osman (1922) transplanted adrenal tissue from a young man who had been killed accidentally to a subcutaneous site in the inguinal region of a man with Addison's disease. The patient's condition was unchanged two weeks later, and he was therefore given an intratesticular homograft of foetal adrenal tissue. The patient improved remarkably, and nine months after transplantation his systolic blood pressure, which before the operation had been 78 mm. Hg, was 115 mm. Hg, and the testicular graft was still palpable. Two years later Pybus (1924) reported two cases of Addison's disease treated by adrenal homografts. One of these patients died a few weeks after operation and it was found on post-mortem examination that the graft had been completely absorbed. The other patient, who received four subcutaneous transplants each consisting of one-half of an adrenal gland from a young man who had been killed in an accident, made a remarkable recovery and six months after operation returned to work as a miner. Two and a half years after operation he relapsed and no trace of the transplant remained, but he improved again after receiving a further homotransplant and was still in good health two years later, though he showed a slight degree of pigmentation.

More cases of Addison's disease showing sustained improvement after adrenal homografting were reported by Halpern and Arkushenko (1927), Reinhart (1928) and Leschke (1929), but, on the other hand, unsuccessful cases were reported by Curschmann (1928), D'Abreu (1933), and Desmarest and Monier-Vinard (1934).

Beer and Oppenheimer (1934) treated two cases of Addison's disease with homografts of adrenal cortical tissue removed in the course of operations on the kidney. Their first patient died and on post-mortem examination only a few of the cells of the graft appeared to be viable. Their second patient improved, but in spite of this was given a second transplant from a different donor two months after the first. Four months later he was still fairly well but he was receiving a high sodium diet and occasional injections of cortical extract, and he relapsed when his sodium intake was reduced.

Bailey and Keele (1935), like Hurst *et al.*, used foetal adrenal tissue, but placed it in the rectus sheath instead of in the testis. Following this procedure the patient's blood pressure, which had been 90/60 mm. Hg, rose in the course of two weeks to 120/80 mm. Hg, and the patient's general condition improved correspondingly. This improvement was still maintained five months later.

Goldzieher and Barishaw (1937) used an adrenal which had been removed from a girl suffering from adrenal virilism to treat a man with Addison's disease. The gland was cut into about 30 thin slices and these were placed in the rectus muscle. The patient regained his strength, lost his pigmentation, and put on weight. He remained well and was able to return to work, despite the fact that he was taking only a normal amount of salt and was not receiving any cortical extract. Six months after transplantation he relapsed, but improved rapidly on being given additional salt and Vitamin C. He relapsed again however and died nine months after the operation. Post-mortem examination revealed broncho-pneumonia. The patient's own adrenals were small and fibrotic, but the transplants contained abun-

dant healthy looking cortical tissue and showed no evidence of either fibrosis or round-cell infiltration

Broster and Gardiner Hill (1946) also transplanted an adrenal from a girl with virilism to a patient with Addison's disease. Instead of using multiple free transplants they placed the whole gland in the rectus sheath and in the hope of providing it with a blood supply they divided the patient's inferior epigastric artery and vein and inserted the proximal end of each of these vessels into the adrenal vein of the transplant. This extraordinary procedure was followed by remarkable improvement and Broster was therefore encouraged to use it in several more patients; unfortunately, however, no histological observations on any of these transplants have been reported.

It seems clear that a patient suffering from Addison's disease may show considerable improvement following homotransplantation of adrenal tissue and that this improvement may be maintained for months or even on occasion for a year or two. It does not necessarily follow, however, that the transplant survives throughout the period of clinical improvement. This is well illustrated by four patients of the author (Woodruff 1953b) each of whom had been under treatment with DOCA and was due for further implants of this material but received instead the minced up adrenal tissue from a 16-20 week old foetus as a homograft to the rectus sheath. Following this procedure cortisone was administered for four weeks in doses ranging from 20 to 100 mg daily with the object of inhibiting the homograft reaction and ACTH was given for five weeks in doses ranging from 10 to 20 mg twice daily in the hope that this might stimulate the transplant and facilitate its survival. All the patients showed striking clinical improvement which was maintained without further treatment for periods ranging from 9 months to a year. Despite this repeated Thorn tests gave no reason to think that the grafts were surviving and in each case biopsy undertaken 7 to 10 months after the operation revealed no trace whatever of the grafts.

Heterotransplants

Jaboulay (1897) used heterotransplants of dog adrenal in two patients with Addison's disease but both died within 24 hours of the operation.

Further cases in which animal adrenal was used were reported by Busch and Wright (1910), Currie (1924), Dmitrijew (1925) and Kanevski (1929).

Busch and Wright used adrenal from a creature described as a female shoat and transplanted it to the testis. Their patient died two and a half weeks later and at autopsy a large amount of cortical tissue was found in the graft.

Currie used sheep adrenal. Suppuration occurred at the site of the transplant but a second gland cut up into small pieces was transplanted and the patient was reported to be well two months later.

Dmitrijew and Kanevski used dog and rabbit adrenal respectively. The grafts were either absorbed or discharged but despite this the recipients showed remarkable improvement and were reported to be free of symptoms one month and nine months after the operation respectively.

The only conclusion that the author can draw from these reports is that clinical improvement is not a reliable criterion of graft survival, especially in a condition such as Addison's disease in which temporary remissions commonly occur spontaneously.

TRANSPLANTATION OF ADRENAL IN PRESENT DAY SURGERY

Patients with gross adrenal deficiency due to Addison's disease or bilateral adrenalectomy can nowadays be maintained in good health by oral administration of cortisone supplemented if necessary by oral adminis-

tration of 9 alpha-fluorohydrocortisone or implantation of desoxycorticosterone. It would be even better if the patient could be given a homotransplant with the certainty that this would survive and function throughout his life, but at present homotransplants fall far short of this ideal, and they are therefore of no therapeutic importance at the present time.

TRANSPLANTATION OF THYROID

HISTORY

Animal Experiments

Early Work

Free transplants of thyroid were studied in dogs by Schiff (1881), Dominici (1891) and Enderlen (1898), and in cats by Eiselsberg (1892, 1898), Enderlen (1898) and Sultan (1898). Schiff used intraperitoneal homotransplants, and removed the recipient's own thyroid (and parathyroids) at a second operation, because he feared that if he performed total thyroidectomy and autotransplantation the animal would die before the graft became established. It was shown by Eiselsberg, however, that it was possible to transplant one lobe autologously and to remove the other one later.

These experiments were undertaken to study the function of the thyroid rather than to yield information about the biology of transplantation. Many of the grafts were too large to survive, and many animals died from tetany, but it was shown that thyroid tissue could survive and proliferate following autotransplantation. This was confirmed by Cristiani of Geneva in rats, rabbits and guinea pigs (Cristiani, 1895, 1900, 1901, 1903a, b, 1905a, b; Cristiani and Cristiani, 1905), and also in reptiles (Cristiani, 1903b), birds (Cristiani, 1904a), and fish and amphibia (Cristiani, 1904b).

Cristiani used one or more small pieces of tissue, described in some experiments (Cristiani, 1903b) as being the size of a millet seed. He obtained his best results in rats, and he found that the external ear was a particularly suitable site which permitted day to day observation of the progress of the graft (Cristiani, 1903a). He made thorough histological studies and observed that the central part of the transplants necrosed but that a process of regeneration occurred, which was similar, he said, to that seen during normal development of the thyroid, in remnants of the gland following incomplete thyroidectomy, and in goitres (Cristiani, 1900).

The success or failure of a graft appeared to depend on many factors. Injection of a local anaesthetic at the graft site had a harmful effect (Cristiani and Ouspensky, 1904; Cristiani and Frigoff, 1905). The degree of thyroid deficiency which was present was of particular importance; thus vascularization and regeneration was more rapid, and subsequent hypertrophy greater, the greater the deficiency which had been created (Cristiani, 1903b), and administration of thyroid orally had a deleterious effect (Cristiani, 1905a). Cristiani thus concluded that a functional demand for thyroid secretion was essential for the success of a thyroid graft.

Cristiani also studied the behaviour of homografts (Cristiani, 1905b) and heterografts (Cristiani, 1904g), and observed that both were unsuccessful. For the most part, however, the homografts were made to intact (non-thyroidectomized) animals and Cristiani attributed their failure to lack of functional demand rather than to the fact that they were homologous. Cristiani and Cristiani (1905) did observe however that the age of the host appeared to have some influence on the behaviour of a homograft,

inflammatory changes being more marked and *regeneration less complete in grafts in* older hosts and this was later confirmed by Tureen (1927) and others

In other experiments Cristiani (1903c 1904b) studied the effect of storage on the behaviour of grafts of thyroid tissue Minute bits of rabbit thyroid exposed to air for more than a few seconds showed a significant loss of vitality whereas they appeared to be unaffected by immersion for 10 minutes in physiological saline at 37°C and were still capable of partial regeneration after immersion for periods up to one hour

Carrel and Guthrie developed a technique for transplanting the thyroid by vascular anastomosis in dogs and in some experiments (Carrel and Guthrie 1905c) they anastomosed the superior thyroid artery to the stump of the superior thyroid vein of the transplant and the superior thyroid vein to the stump of the artery An autograft of the whole gland transplanted in this way was exposed for examination at intervals and 58 days after the operation appeared normal except for the fact that it was slightly enlarged

Further Experiments with Autotransplants

Further experiments with free autotrans*plants of thyroid tissue were performed by* Payr (1906) Salzer (1909) Carraro (1909a b) Manley and Marine (1915) Kummer (1917) Hess and Strauss (1917) Loeb and Hesselberg (1919) Loeb (1926d e) Iwao and Tsumaki (1932) Marine and Rosen (1934) Salmon and Severinghaus (1936) Williams (1937 1939) Bisgard (1938) Otsuka (1938) Ingle and Cragg (1939) Gabe and Arvy (1947) Woodruff and Woodruff (1950) Desaive Mewissen and Closon (1951) Bondy (1951) Cordier Craps and Martin (1951) Hamolsky and Gierlach (1952) Rupp (1952) and others Various sites were used for the transplants including the subcutaneous tissue the peritoneal cavity and omentum the spleen the thyroid capsule the brain the anterior *chamber of the eye and the bone marrow*

Some of these experiments were performed in order to study the control of thyroid secretion and it was established (Manley and Marine 1915 Kummer 1917 Marine and Rosen 1934) that thyroid tissue could function in the absence of nervous connexions but was subject to hormonal control Smith (1914) found that oral administration of 0 2 g potassium iodide daily had no influence on the regeneration of thyroid autografts in guinea pigs but Manley and Marine (1915) on the other hand reported that administration of iodine caused thyroid grafts in rabbits to regress Loeb and Hesselberg (1919) studied the effect of different grades of thyroid deficiency and concluded contrary to Salzer (1909) and others that the degree of deficiency did not influence the regeneration of autografts though it did to some extent determine whether regeneration was followed by hypertrophy More recently however Woodruff and Woodruff (1950) found that in the special case of grafts in the anterior chamber of the eye the degree of thyroid deficiency was of decisive importance unless the animal bearing the graft received injections of pituitary extract rich in thyrotrophin

Other experiments have been under*taken to determine whether the thyroid* hormone is destroyed or inactivated by passage through the liver Gabe and Arvy (1947) in the light of histological examination of the pituitary liver and adrenals of rats bearing thyroid autografts in the spleen or mesentery and of the grafts themselves concluded that thyroid hormone was completely removed by the liver Cordier Craps and Martin (1951) however from histological examination of intrasplenic grafts alone concluded that their secretion was only partly inactivated while Bondy (1951) and Rupp (1952) observed that such grafts in immature animals retained their normal

structure and maintained normal growth,* and therefore concluded that no inactivation occurred. Experiments using radioactive iodine have also given conflicting results. Desaive, Mewissen and Closon (1951) found in rabbits that intrasplenic thyroid autografts which had completely regenerated showed a normal uptake of I^{131}, suggesting that their secretion was not inactivated.† Hamolsky and Gierlach (1952) on the other hand, in more elaborate experiments, observed that the level of protein bound I^{131} in the serum of thyroidectomized rats bearing intrasplenic grafts 24 hours after injection of 15 μc. carrier free I^{131} was intermediate between the values obtained in thyroidectomized animals without grafts and in thyroidectomized animals with grafts outside the portal venous territory. They took special care to exclude animals in which the thyroidectomy was incomplete, and concluded that the secretion of the intrasplenic grafts was partly inactivated. It seems likely that this conclusion is correct, and that the lack of agreement is due partly to unrecognized incomplete thyroidectomy and partly to vascular adhesions allowing intrasplenic grafts to drain partly to systemic veins

Thyroid autografts have also been observed in transparent chambers in the rabbit ear (Williams, 1937, 1939), and the formation of new follicles has been studied in detail.

Further Experiments with Homotransplants

Cristiani, as we have seen, seems to have assumed that autotransplants and homotransplants would behave in the same way, and when he observed a difference he invoked various factors to explain it such as the age of the recipient or the absence of functional demand. Stich and Makkas (1908), who studied transplants of thyroid by vascular anastomosis in dogs, showed however that homotransplants always necrosed or were absorbed completely whereas autotransplants were usually successful, and this was confirmed by Goodman (1916), Kawamura (1919) and Shafiroff and McCloskey (1937); while Smith (1911), from a critical study of the literature, concluded that free homotransplants were also destroyed however perfect the technique, probably, he suggested, in consequence of "a biochemical difference in the tissue of different animals of the same species."

Leo Loeb and his colleague Cora Hesselberg carried out numerous experiments in guinea pigs and other animals (Hesselberg, 1915; Loeb, 1918a, b, 1926d, e, 1927; Hesselberg and Loeb, 1918; Loeb and Hesselberg, 1919) and reached much the same conclusion. Loeb described the histological features of the homograft reaction, and reported that the intensity of the reaction, and the rapidity with which a homograft was destroyed, were less when the donor and host were close relatives than when they were unrelated. He postulated the existence of what he called *individuality differentials,* and though this concept appears at times a little nebulous it seems to correspond in the main with the biochemical differences of Smith. As we have seen (p. 70) Loeb and Hesselberg studied the behaviour of successive transplants in the same host, but they failed to observe any significant difference between them, and concluded in consequence that immunological factors played little or no part in the destruction of homografts. This conclusion however is unjustified, because in the experiments cited the transplants were obtained from different donors, and it is in fact erroneous (Woodruff and Woodruff, 1950).

While it is true that thyroid homografts between genetically dissimilar animals are

*Bondy observed also that the level of protein-bound iodine in the serum remained normal in three thyroidectomized rats bearing intrasplenic thyroid autografts.

†If inactivation occurred the grafts might be expected to be hyperactive in consequence of increased secretion of thyrotrophin by the pituitary.

usually soon destroyed many exceptions to this rule have been reported

In the first place homografts from both immature (May, 1932, 1936, Dameron, 1950, Krementz, 1952) and adult (Goldner and Frazin, 1937, Bisgard, 1938, Woodruff and Woodruff, 1950) donors become vascularized, and may survive indefinitely, in the anterior chamber of the eye, and in some experiments evidence of functional activity has been sought for and obtained The immunological implications of this phenomenon were investigated in some detail by Woodruff and Woodruff (1950) and their findings have been considered in Chapter 5

Secondly, May (1934) has reported that in rats subcutaneous homografts of thyroid from new born donors in thyroidectomized hosts not only survive but show the structure of normal adult thyroid after periods ranging from 9 to 16 months

Thirdly, thyroid tumours may survive homotransplantation Bielschowsky, Griesbach Hall, Kennedy and Purves (1949) induced thyroid tumours in rats by prolonged administration of methyl thiouracil, with or without prior administration for a few days of 2 acetamidofluorene, and showed that these could often be successfully transplanted into thyroid deficient hosts of the same inbred — but by no means genetically uniform — strain In one instance what had been a well differentiated adenoma became transformed on serial transplantation in thyroid deficient hosts into a highly anaplastic carcinoma which was transplantable in rats without thyroid deficiency (Purves, Griesbach and Kennedy, 1951)

Fourthly, it has been shown by Woodruff and Sparrow (1958) that thyroid homografts survive and function indefinitely in hosts made tolerant by injection during early life of cells from the prospective graft donor This phenomenon has been discussed in Chapter 7

Finally, many investigators, including Woodruff and Woodruff (1950), have observed that subcutaneous or intramuscular homografts of adult thyroid sometimes survive for unexpectedly long periods, and so far no satisfactory explanation for this phenomenon has been put forward.

Further Experiments with Heterotransplants

Bisgard (1938) studied the behaviour of heterografts of dog and human thyroid in rabbits In every instance the grafts were rapidly destroyed

Tests of Functional Activity

Mention has already been made of attempts to assess the functional activity of thyroid grafts, but it will be convenient to summarize here the methods which have been used These are as follows

1 Histological study of the graft, and of the pituitary and other endocrine tissues of the host (Gabe and Arvy, 1947, Cordier, Craps and Martin, 1951). A high degree of functional activity is indicated by the presence of tall columnar cells lining the acini of the graft, and by a relative increase in the number of basophils and decrease in the number of eosinophils in the host's pituitary

2 Observations on the growth rate of thyroidectomized immature hosts bearing thyroid grafts (Bondy, 1951).

3 Chemical estimation of the protein bound iodine in the serum of the host (Bondy, 1951)

4 Uptake of radioactive iodine Three different procedures have been used Desaive, Mewissen and Closon (1951), and Bennett and Gorbman (1951), removed the graft shortly after giving an injection of I^{131} and prepared autoradiograms Hamolsky and Gierlach (1952) measured the amount of protein bound I^{131} in the serum following injection of this isotope, and Woodruff and Sparrow (1958) made counts over the grafts using a collimated scintillation counter This last method is simple to use if the appropriate equipment is available, and

gives excellent results. Moreover, as Woodruff and Sparrow showed, it is possible by making serial counts to obtain an estimate both of the volume of functioning thyroid tissue and of the functional activity per unit volume.

Clinical Observations

Theodor Kocher of Berne in 1878 began using thyroid grafts clinically in patients with thyroid deficiency (cited A. Kocher, 1923), and subsequently (Kocher, 1908, 1914a, b) published a number of papers reporting his results. He first used heterografts from sheep and goats, and later, from 1883 (cited Horsley, 1890), homografts from patients with either primary Graves' disease or nodular toxic goitre. The usual site for the grafts was the medullary cavity of the upper end of the tibia, but some were placed in extraperitoneal tissue, in a pocket in the parietal peritoneum, or in the spleen. Some of the heterografts were reported to give good results, especially when the tissue was obtained from a pregnant animal, but the homografts were found to be more reliable.

Lannelongue (1890) used a heterograft of sheep thyroid in one patient, and further cases of heterografting were reported shortly afterwards by Bettencourt and Serrano (1890), Merklen (1890), Wölfler (1891), Robin (1892), Macpherson (1892), Gibson (1893) and von Gernet (1894). Many of the recipients showed temporary improvement, but relapsed within a month or two. Some apparently more successful cases of heterografting were reported, for example by Mamourian (1914), who transplanted sheep thyroid to a four year old cretin and observed a remarkable improvement in growth, appearance and mentality, but this procedure was soon abandoned, and it seems clear that any beneficial results can only have been due to the release of thyroid hormone actually in the graft at the time of the operation.

Bircher (1890) used a homograft in a girl who had been subjected to total thyroidectomy (by mistake!) and had developed severe myxoedema. The graft was placed in the peritoneal cavity and the patient was markedly improved for about three months and then relapsed. She improved after a second graft and menstruation was reestablished, but her condition began to deteriorate again after about a year.

Further trials of homografts by other German surgeons were discouraging, but Cristiani and his colleagues (Cristiani, 1904d, e, f; Gautier and Kummer, 1905; Charrin and Cristiani, 1906; Cristiani and Kummer, 1906) used multiple subcutaneous homografts, introduced in some cases by means of a trocar and cannula or a wide bore needle, in a large number of patients with myxoedema or cretinism, and reported some excellent results. One graft (Cristiani, 1904c) which was removed for histological examination after two months contained recognizable thyroid tissue but showed a considerable inflammatory reaction, in striking contrast to two experimental autografts which showed normal thyroid tissue with no inflammatory reaction after 6 months and 15 months respectively; the possible significance of this difference, however, does not seem to have occurred to Cristiani.

Payr (1906) used an intrasplenic homograft from the mother in a cretin, and reported that there was marked improvement in the child's general health and mentality which persisted for two years.

Homografts continued to be used for many years, especially on the continent. For the most part free transplants were used (Bircher, 1909; Bramann, 1909; Groves and Joll, 1910; Eiselsberg, 1915; A Kocher, 1923; Madureira, 1928; Le Fort, 1937; Voronoff, 1937; Kubanyi, 1941; and others), and they were placed in various sites including muscle (Groves and Joll, 1910; Le Fort, 1937), bone marrow (Kocher,

1923) the peritoneal cavity (Kocher 1923) the sympathetic nerve plexus around the internal carotid artery (Kubanyi 1941) and the remnant of the host s thyroid after subtotal thyroidectomy (Giangrasso 1947) but Bogoras (1926) and Portugalow (1929) made transplants by vascular anastomosis

Eiselsberg (1915) identified hypertrophic thyroid tissue in one case after homografting though at some distance from the site of the graft but for the most part the success of the operation was judged by clinical improvement unsupported by examination of the graft site In some cases for example that of Groves and Joll (1910) it appears almost certain that the clinical improvement was psychogenic but it does not seem possible to account for all the results in this way

The largest series of cases was reported by Albert Kocher (1923) and comprised ten patients with total absence of the thyroid gland three of whom obtained considerable benefit from the operation and 204 patients with various degrees of hypothyroidism 53 of whom were reported as cured 43 as so much improved that they required only occasional small doses of thyroid by mouth 79 as improved and requiring regular but small doses of thyroid and 29 as unimproved Kocher concluded that the transplants functioned for at least several years He thought that in most cases the patient s own thyroid resumed function and when it appeared that this had not happened he made a second a third and in one case a fourth transplant

The longest follow up was reported by Le Fort (1937) His patient was a 2½ year old cretin who received an intramuscular homograft consisting of about a third of one lobe of a thyroid from a guillotined criminal This mode of execution was clearly not designed to facilitate thyroid grafting but Le Fort succeeded in obtaining a satisfactory graft and transferring it to the patient within half an hour of the donor s death Within a few days the appearance of the patient was transformed and she began to show signs of intelligence She continued to improve for some years but thereafter remained more or less stationary and when 14 years old resembled in physical and mental development a child of nine

Stone Owings and Gey (1934b) used multiple small homotransplants of thyroid which had been cultured for about two weeks in a medium containing serum from the prospective recipient in five patients with thyroid deficiency including two cretins In no case however had sufficient time elapsed at the time their paper was written to show whether the grafts were successful and no subsequent report appears to have been published

Mandl (1943) attempted to treat spondylarthritis ankylopoietica by homografts of thyroid from patients with toxic goitre He claimed that the transplants remained active for 6 11 months in three patients he studied but this opinion was based on observations of the basal metabolic rate and no histological studies were carried out

Swan Harper and Cristensen (1952) reported an interesting case in which they made autografts following the removal of a lingual thyroid in a 7 year old girl At the time of the operation exploration of the neck revealed no thyroid tissue at the normal site and part of the tissue removed from the tongue was therefore replaced in the form of free grafts to the pectoral and rectus muscles Another portion of the same tissue was stored for two months at 4° C in Ringer s solution containing 10 per cent human plasma and subsequently transplanted to the liver Five months after the original operation the patient was grossly hypothyroid and homografting by vascular anastomosis was attempted though without much optimism During the next few months the patient s condition improved and nine months after the initial autotransplantation studies with radioactive iodine

which had hitherto been negative, revealed a significant (20 per cent) uptake, and monitoring showed that all the activity lay in the intramuscular autografts. It was thus apparent that these had taken, but that there had been a six to nine months' delay before they had begun to function. At this stage the patient was given thyroid by mouth (1 gr./day), and subsequent studies with radioactive iodine showed that function of the grafts had greatly diminished. The grafts became more active again when administration of thyroid was suspended, but were not adequate to maintain the patient in a euthyroid state, and administration of thyroid by mouth was therefore resumed. Three years after the operation the child was short in stature and radiographs of the long bones showed that her "bone age" was well below her chronological age. The I^{131} uptake was normal but did not increase following administration of thyrotrophin. It was therefore concluded that the homografts had survived and were functioning, but had not grown at a rate commensurate with the growing needs of the patient (Swan, 1958). .

Szilagyi, Barret, Longabaugh and Preuss (1953) made experimental autografts of thyroid to the sternomastoid muscle in nine patients following total, subtotal or hemithyroidectomy. Counts were made over the grafts following administration of 25 μc. I^{131} by mouth, and observations were also made of the protein bound iodine and cholesterol levels in the serum, and the basal metabolic rate. The transplants consistently took, and their functional activity steadily increased with time. This increase in activity was attributed, at least in part, to actual growth of the grafts. The rate and magnitude of the increase in function depended on the type of tissue transplanted, grafts from hyperplastic goitres showing the greatest growth potential.

TRANSPLANTATION OF THYROID IN PRESENT DAY SURGERY

It would seem reasonable, after removing a lingual thyroid, to replace all healthy looking thyroid tissue in the form of autografts unless exploration of the neck reveals an adequate amount of thyroid tissue in the normal site, but the opportunities for performing this operation will arise only very occasionally.

In our present state of knowledge there are no indications for using thyroid homografts, and this applies even more strongly to heterografts, because thyroid deficiency can be treated far more satisfactorily by hormone replacement therapy, using dried thyroid or, better still, thyroxin-sodium. Indeed, even if it were possible to make thyroid homografts capable of functioning for an indefinite time, most patients would probably prefer to take a daily pill rather than submit to an operation, particularly one involving transplantation of tissue from some other person.

TRANSPLANTATION OF PARATHYROID

HISTORY

Animal Experiments

Cristiani and Ferrari (1897) appear to have been the first to observe the persistence and regeneration of grafts of parathyroid. They were concerned to prove that the parathyroid glands were distinct entities, and that parathyroid tissue was not simply, as some believed, undeveloped thyroid tissue. The species of animal and type of graft are not stated, but it seems likely that the experiments were performed in rats and that the grafts were autologous.

Subsequently, successful autotransplantation of parathyroid was reported not only

in rats (Cristiani 1905a Leischner 1907) but also in cats (Cristiani 1905c Biedl 1907) and dogs (Wallbaum 1903 Biedl 1907 1908 Halsted 1907 1909 1912 Pfeiffer and Mayer 1907 Landois 1911) As a rule the tissue was transplanted without delay but Cristiani (1905d) observed that parathyroid tissue was more resistant than thyroid to storage in saline blood or serum and could be successfully transplanted after being kept in physiological saline for between half an hour and an hour

The grafts were placed in various sites including the external ear (Cristiani 1905c skeletal muscle (Leischner 1907 Halsted 1909 1912) extraperitoneal tissue Leischner 1907) the spleen (Biedl 1907 1908 Halsted 1907) and the thyroid gland Halsted 1907) In addition Landois (1911) injected minced parathyroid tissue into the external jugular vein As a rule he allowed the material to enter the circulation and it was presumably carried to the lungs but in some experiments the vein was ligated proximal and distal to the site of injection

The success of the grafts was judged partly by histological examination and partly by their capacity to prevent the development of tetany in hosts lacking other parathyroid tissue It was found that a very small piece of parathyroid tissue sufficed for this purpose In one of Halsted's (1912) experiments for example the graft was removed from a dog after 12 months and was found to consist of a minute piece of parathyroid one quarter of a millimetre in diameter surrounded by thyroid tissue The animal which as far as was known had no other parathyroid tissue had been maintained in good health but developed tetany five weeks after removal of the graft and died some seven weeks later It is noteworthy that grafts in the spleen appeared to be functionally just as effective as those in other situations (*see* e g Biedl 1908)

Halsted (1907 1909) stated that parathyroid autografts were successful only if the animal had been rendered markedly parathyroid deficient * This assertion gave rise to the general hypothesis sometimes known as Halsted's principle (p 25) As we have seen this hypothesis though by no means universally true holds good within limits for some endocrine grafts and especially for those in the anterior chamber of the eye The evidence on which Halsted based his assertion is however quite unconvincing and the observation of Shambaugh (1936) that the survival of parathyroid autografts in dogs is not prejudiced by administration of large amounts of either parathormone or viosterol makes it appear unlikely that the principle applies to grafts of this tissue

The local effect of parathyroid tissue on bone was studied by Barnicot (1948) and Chang (1951) in mice and rats using autografts or intrastrain homografts of parathyroid in juxtaposition with autografts of bone They observed bone absorption associated with osteoclastic activity beginning about 10 days after the transplantation

Parathyroid homografts between genetically dissimilar animals have with few exceptions been unsuccessful Camus (1905) found that homografts to the external ear of the rabbit were destroyed within six weeks to three months and the degree of parathyroid deficiency in the host made little or no difference to the fate of the graft Halsted (1909) reported that homografts of parathyroid to the rectus muscle in dogs became necrotic or were absorbed in a period ranging from a few days to three weeks and failed to prevent the development of fatal tetany in parathyroidectomized hosts Subsequently Landois (1911) showed that homografts in the form of intravenous injections of minced parathyroid were similarly ineffective The observation of Iselin (1908) that tetany in parathyroidectomized rats could be partly controlled by repeated

*He reported success in 61 per cent of dogs in which at least half the animal's parathyroid had been removed

homografts to the spleen is of little significance in the present context because it seems likely that the grafts acted simply as depots of parathormone. The work of Joannovics (1911) in cats suggests that homografts of foetal parathyroid may on occasion function for a few weeks, but they too are eventually destroyed.

Homografts in the anterior chamber of the eye, as might be expected, appear to behave somewhat differently. Richter and Eckert (1937a), who studied such grafts in rats, found healthy parathyroid tissue in two out of six animals 37 days after grafting. As evidence that the grafts were functioning Richter and Eckert pointed to the fact that although the recipients had been subjected to total parathyroidectomy they did not show any abnormal appetite for calcium when offered calcium lactate solution as an alternative to water.

Clinical Observations

Free homografts of parathyroid have been used with apparent success in patients with tetany by many investigators, including Garrè (1907), Eiselsberg (1908), Brown (1911), Wilmoth (1931), Stone, Owings and Gey (1934a, b), Peycelon and Guillemin (1946), Kooreman and Gaillard (1950), and Gaillard (1954). The phrase "apparent success" is used advisedly because in no case was survival of the graft proved by biopsy. Most of the patients had developed tetany following thyroidectomy for goitre, and this complication often ceases spontaneously, while in the remainder the evidence for the existence of parathyroid deficiency is not altogether convincing.

Stone, Owings and Gey (1934a, b) cultured the tissue prior to grafting in a medium containing serum from the prospective host, and then transplanted multiple small fragments about a millimetre in diameter to the vicinity of the main blood vessels in the axilla or groin, and they suggested that this technique helped to determine the successful outcome. More recently Gaillard and his colleagues (Kooreman and Gaillard, 1950; Gaillard, 1954) have used parathyroid from foetuses or infants dying soon after birth, again after cultivation in a medium containing serum from the prospective host. In 1954 Gaillard reported 30 cases treated in this way. Seven appeared to be cured, 2 showed improvement but were not free from symptoms, 19 had obtained no benefit from the operation, and in 2 the result was unknown. Once again no biopsies were performed, and the significance of the apparently successful cases is therefore uncertain.

There have also been reports of several cases of parathyroid deficiency treated by homotransplantation by vascular anastomosis of the thyroid and parathyroids from an infant, together with the superior thyroid artery and a segment of the carotid, and the middle thyroid vein and a segment of the internal jugular vein, on each side. Three of these cases have been treated by Sterling (1958), one by Jordan, Foster and Curd (1958) and two by Nicks (cited Conway, 1958). Three of them are apparently doing well after periods of time ranging from nine months to four years after transplantation, but it is noteworthy that in one of Nicks' patients exploration one year after transplantation revealed no trace of the graft.

Even though a parathyroid homograft is destroyed it may for a time act as a depot for parathormone. As long ago as 1907 Pool suggested that this fact, coupled with the fact that sooner or later spontaneous recovery often occurs in patients with postoperative tetany, accounted for the apparent success of parathyroid grafting, and by way of illustration he reported one case in which subcutaneous homografts consisting of five parathyroid glands, obtained after death from three different donors, had been used. An even more striking example was reported more recently by Bruce and Strong (1955). The patient was a boy aged four who

had had symptoms of severe parathyroid deficiency since he was a year old and was under treatment with calciferol His mother had a parathyroid tumour and when this was removed pieces weighing 1 11 g were transplanted to the medullary cavity of the upper end of the child s tibia and other pieces weighing 2 34 g were transplanted to the child s rectus abdominis muscle Within a few days the child s serum calcium had risen to over 15 mg /100 ml and removal of the transplants was contemplated but the urinary output then increased and the serum calcium fell sharply Five weeks after transplantation the serum calcium had fallen to 8 5 mg /100 ml and treatment with a high calcium low phosphorus diet and calciferol which had been temporarily suspended had to be resumed

Heterotransplants of parathyroid may also act for a short time as hormone depots and this almost certainly explains the temporary improvement observed by Brown (1911) Wilmoth (1931) and others in patients with post-operative tetany who received such grafts

TRANSPLANTATION OF PARATHYROID IN PRESENT DAY SURGERY

If after sub total thyroidectomy one or more parathyroid glands can be seen in the specimen they should be cut into small pieces and transplanted to the sternomastoid muscle Many surgeons appear to be sceptical of the value of this procedure but without any good reason

Homografts and heterografts of parathyroid in the author s view have no place in present day surgery If the homograft problem is solved however, homografts of parathyroid will be of considerable value because parathormone replacement therapy is far from satisfactory

TRANSPLANTATION OF PANCREATIC ISLET TISSUE

Many surgeons have thought wistfully of the possibility of treating diabetes mellitus by transplants of pancreatic islet tissue

Transplants of whole pancreas were used in a few diabetic patients before insulin became available but as might be expected were completely unsuccessful

More recently attempts have been made to grow islet tissue in culture for use in homografting operations Selle (1935) cultured foetal dog pancreas and also pancreas from adult dogs in which the pancreatic duct had been ligated 10 weeks previously in order to cause degeneration of acinar tissue in a medium containing Tyrode s solution beef extract and homologous serum Subsequently 30 to 50 transplants were made to the neighbourhood of large vessels in the axilla or groin of the dog which had provided the serum for the culture medium In all 10 dogs received grafts 5 of them intact animals and 5 animals which had previously been subjected to partial pancreatectomy In every case the grafts were absorbed within three weeks

Murray and Bradley (1935) cultured two human islet cell adenomas by the hanging drop technique in human plasma with heterologous spleen extract For the first 7 10 days the plasma was obtained from the patient from whom the tumour had been taken after which plasma from a diabetic patient was used instead Cultures of the first tumour became infected and the procedure had to be abandoned after 5 weeks The second tumour was maintained successfully for 6½ weeks and fragments from it were then transplanted by the technique of Stone *et al* (1934a b) to the patient whose serum had been used for the culture medium Following the transplantation the patient was maintained on virtually the

same dosage of insulin as previously and showed no significant change in the level of sugar in the blood or urine.

Swan and Rundles (1957) transected part of the pancreas in dogs and transplanted it, with its blood vessels intact, to an accessible part of the abdominal wall. Subsequently they divided the pedicle and removed all the remaining intra-abdominal pancreas. Later still they removed the portion of the abdominal wall containing the graft, cut it into thin slices and implanted these as autografts in a different site in the abdominal wall. In a number of animals diabetes, which had developed after the second stage of the procedure and had had to be treated by administration of insulin, improved following the third procedure to such an extent that insulin could be withdrawn completely after a period of 30-60 days. It thus appeared that the grafts were functioning, and this was confirmed by the fact that the diabetes recurred when the grafts were removed. Unfortunately, however, islet tissue could not be definitely identified on histological examination.

Two conditions which are clearly necessary for further progress along these lines are the development of satisfactory methods of culturing islet tissue and a solution of the clinical homograft problem. It is by no means certain, however, that these conditions are sufficient, because it may well be that the environment of the diabetic patient is in some special, but as yet undefined, way unfavourable for islet tissue, and might therefore prevent the graft from surviving, or from functioning properly if it did survive.

CHAPTER 24

Transplantation of the Gonads

TRANSPLANTATION OF OVARY

HISTORY

Animal Experiments

Early Work

Experimental ovarian transplantation dates from about the end of last century. Priority appears to belong to Knauer (1896, 1897, 1898a, b, 1899, 1900), who studied ovarian transplants to the broad ligament, and also to the abdominal wall in rabbits. Autotransplants* persisted indefinitely and showed evidence of functional activity. Ovulation occurred regularly, and it was shown that the expelled ova were capable of giving rise to pregnancy under appropriate conditions. In addition, the transplants prevented atrophy of the uterus and other parts of the genital tract following bilateral oophorectomy. Homotransplants* behaved quite differently, and although Knauer considered that homotransplants could be made to function there was no convincing evidence that they did so in his experiments.

Experiments by various other investigators in rabbits (Grigorieff, 1897, Arendt, 1898, Marchese, 1898, Fish, 1899, McCone, 1899, Preobrashenski, 1899, 1900, Rubinstein, 1899, Herlitzka, 1900, Basso, 1905, Uffreduzzi, 1911), guinea pigs (Ribbert, 1898, Halban, 1900, 1901; Herlitzka, 1900, Schultz, 1900, 1902, Castle and Phillips 1909, 1911, 1913, Uffreduzzi, 1911), and rats (Marshall and Jolly, 1905, 1907, 1908) on the whole confirmed these findings. Some long surviving homotransplants were reported (Grigorieff, 1897, McCone, 1899, Basso, 1905), and Magnus (1907) found that rabbits whose ovaries were removed and replaced by homotransplants often became pregnant when mated, but it is difficult to assess the reliability of the observations.

Foa (1900, 1901) appears to have been the first to study homotransplants of foetal ovarian tissue. In experiments in rabbits he found that grafts to hosts which were 1-2 weeks old underwent maturation, but began to atrophy after 90-170 days and were finally completely destroyed, while in older hosts the period of survival was somewhat shorter.

During this period a few experiments with ovarian autotransplants and homotransplants were performed in large animals, including dogs (Marchese, 1898, Nattrass, 1910, Libroia, 1911), sheep (Amico-Roxas, 1901, Voronoff, 1913) and monkeys (Halban, 1901, Marshall and Jolly, 1908).

Heterotransplants were studied by McCone (1899), who transplanted ovary from dog to rabbit, and Lukaschewitsch (1901).

From these beginnings a vast literature has developed, much of it, however, is of little value because many papers fail to state clearly the conditions of the experiment

*Knauer refers to transplantation to another site in the same animal as homotransplantation and to transplantation from one animal to another of the same species as heterotransplantation but we shall adhere to the terminology used elsewhere in this book.

and many others report observations which cannot be reproduced or conclusions which appear to be based on a misinterpretation of the findings presented. It would be a tedious and almost certainly an unrewarding task to review this work in detail,* and we shall confine our attention to those facts which appear to have been definitely established, and their possible implications.

Criteria of Survival

Great care is required in the assessing of survival of ovarian grafts because in some species, notably the mouse (Parkes, Fielding and Brambell, 1927), there may be a striking degree of regeneration of ovarian tissue after apparently complete bilateral ovariectomy, and it seems likely that a considerable number of the reported instances of prolonged survival of ovarian homografts and heterografts in the ovarian capsule of the host (*see* e.g. Magnus, 1907; Voronoff, 1913; Guthrie and Lee, 1915; Whitney, 1916) are erroneous on this account.

The criteria which have been used are as follows:

1. Evidence of endocrine function in the graft.
2. Ovulation and pregnancy in spayed hosts.
3. Visible cyclic changes in grafts in the anterior chamber of the eye.
4. Histological evidence.

Ovarian grafts may, under appropriate conditions, produce a variety of hormones including oestrogens, progesterone, androgens, and possibly adrenocortical-like hormones.

Oestrogenic activity may be indicated in oophorectomized hosts by prevention of atrophy of the uterus and other parts of the genital tract (*v. supra*), stimulation of the mammary gland (Gardner, 1935), and restoration of oestrus in rats (Marshall and Jolly, 1905, 1907, 1908; Pfeiffer, 1934) and guinea pigs (Adamberg, 1930), or menstruation in monkeys (Halban, 1901; Herrick, 1928; Mandl and Zuckermann, 1949) Under certain circumstances, notably with grafts to the tail, continuous oestrus may be produced in some species (Hernandez, 1943; Bielschowsky and Hall, 1953).

The capacity of ovarian grafts to produce progesterone is suggested by the observations of Herrick (1928), and Nelson and Haterius (1930), in guinea pigs and rats respectively, that pregnancy may continue after bilateral oophorectomy in the presence of a viable ovarian graft.

Androgenic activity may be manifested in male hosts by prevention of the usual changes in the genital tract (Hill, 1937a, b, 1941; Deanesly, 1938; Hill and Strong, 1940; Katsh, 1950) or adrenal cortex (Idzkowsky and Starkey, 1942) which occur after castration, and in adult female hosts by the occurrence of atrophy of the genital tract (Lampton and Miller, 1940) Androgenic activity has been demonstrated with subcutaneous grafts to the external ear in mice (Hill, 1937a, b, 1941; Hill and Strong, 1940; Idzkowsky and Starkey, 1942), and to the ear and other sites in rats (Deanesly, 1938; Hernandez, 1943); in addition, local androgenic effects have been demonstrated with grafts to the seminal vesicle (Katsh, 1950). According to Hill the type of hormone produced probably depends on the temperature of the graft, and in mice androgenic effects are much more pronounced when the animal is kept in a cool (22°C.) environment and the temperature of the graft is in consequence lower than that of the normal ovary. Lampton and Miller (1940) concluded that the same was true in rats, but Deanesly (1938) had previously reported that this was not the case.

Hill (1948) also obtained evidence that ovarian grafts to the external ear or testis might enable the host to survive much longer than normal after bilateral adrenalec-

*Anyone attempting to do so should consult the reviews by Martin (1908, 1911, 1915, 1917, 1922) and Marshall (1922), and the bibliography prepared by Krohn (1955d).

tomy and concluded therefore that ovarian grafts are capable of producing adrenocortical-like hormone

Much work has been done on the endocrinological status of ovarian grafts having their venous drainage to the portal circulation A graft of this kind in a hare was studied as long ago as 1907 by Foges and subsequently further experiments were made in dogs by Romberg (1935 1938) but were of no special interest until it was shown by Golden and Severinghaus (1938) that oophorectomized rats bearing ovarian autografts in their spleens remained permanently anoestrous A few years later Biskind and Biskind (1944) made the important observation that granulosa-cell tumours developed in intrasplenic ovarian autografts in oophorectomized rats and they suggested that this was the result of inactivation of oestrogens from the graft by the liver with consequent excessive secretion of pituitary gonadotrophin Confirmatory evidence was provided by Li and Gardner (1947) who observed the development of similar tumours in intrapancreatic ovarian grafts in oophorectomized mice and by the Biskinds (Biskind and Biskind 1948 Biskind Kordan and Biskind 1950) who showed that intrasplenic ovarian autografts atrophied if one host ovary was left in its normal place but showed proliferative changes when this was removed

The tumours observed by Biskind and Biskind and by Li and Gardner did not metastasize but Furth and Sobel (1947) succeeded by serial transfer of a granulosa-cell tumour of the Biskind type in pure line mice in producing two transplantable metastasizing tumours and subsequently Li (1948) obtained one metastasizing tumour in an intrasplenic ovarian graft in a castrated male mouse which received weekly injections of progesterone

Li and Gardner (1950) reported that ovaries from old donors to young hosts of the same strain were as susceptible to tumorigenesis as young ovaries but Klein (1952) subsequently recorded a higher incidence of tumours in grafts from young donors Biskind Kordan and Biskind (1950) and Guthrie (1954) studied the changes which precede tumour formation and Biskind and Kordan (1949) studied the effect of pregnancy on intrasplenic ovarian grafts

The conclusion which has been drawn from all this work is that oestrogens are inactivated by passage through the liver This appears to be very largely true in rodents though Bernstorf (1951) has produced evidence that it is not completely true at any rate in mice In monkeys however van Wagenen and Gardner (1950) observed menstruation and other evidence of ovarian function in oophorectomized animals bearing intrasplenic ovarian autografts* It would seem therefore that in the monkey oestrogens are not inactivated by the liver or at any rate are inactivated only to a very limited extent

The capacity of ovarian grafts to ovulate and for the liberated ova under certain conditions to become fertilized has been used not only as a criterion of graft survival but as a tool in genetic research (Chapter 10) The early work of Guthrie (1907b 1911) in hens and Castle and Phillips (1909) in guinea pigs suggested that the characteristics of the offspring were influenced by the genetic constitution of the host animal and not only as might be expected by that of the graft donor and the male parent after further investigation (Castle 1911 Castle and Phillips 1913) however it appeared that this conclusion was probably erroneous and that the findings on which it was based were the result of regeneration of host ovary

After a lapse of many years the question was reopened largely owing to the work of Robertson and Russell Robertson (1940 1942 1945) transplanted one ovary from a

*Referred to by van Wagenen and Gardner as homografts

yellow mouse of an inbred line (101A') into an agouti litter mate differing from the donor in but a single genetic characteristic. Twelve out of 18 hosts which had been completely oophorectomized gave birth to yellow offspring when mated with an agouti male, and since the yellow colour is dominant it seems clear that these must have developed from ova liberated by the grafts. Russell and his colleagues (Russell and Douglass, 1945, 1946; Russell and Hurst, 1945) transplanted ovaries from either adult or embryo mice of an inbred strain to F1 hybrids resulting from the mating of a chinchilla varient of the donor strain with a mouse of a different inbred strain. In several instances offspring were produced which, it appeared, could come only from the transplanted ovaries, and it was therefore possible to compare the effect of different maternal environments on the development of genetically identical embryos. Subsequently, as an extension of this work, Russell and Gover (1950) succeeded in obtaining offspring from transplanted ovaries of foetal mice homozygous for a lethal gene that normally kills before birth.

One of the difficulties with experiments of the kind we have been considering is that scarring may occur after the transplantation and prevent the passage of ova from the graft to the Fallopian tube and uterus. In some species, notably mice, much better results can be obtained, as Parrott and Parkes have shown (Parrott and Parkes, 1956a, b, Parrott, 1958b), by destroying the host's ovaries by irradiation, instead of removing them surgically, prior to orthotopic transplantation.

The use of F1 hybrids of the donor and another strain as recipients, in order to avoid a homograft reaction, imposes a serious restriction and, as we have seen (Chapter 7), a possible alternative is to use as hosts animals made specifically tolerant of donor strain tissue. Another procedure is to study the behaviour of fertilized eggs transferred to a foreign homologous host, as described in Chapter 10.

The occurrence of visible cyclic changes, notably in the degree of vascularization, in grafts located in the anterior chamber of the eye, provides striking evidence of graft survival. Such grafts react to the administration of gonadotrophin, and this has been used as the basis for a clinical diagnostic pregnancy test by Allen and Priest (1932), Abramowicz and Zaleski (1935), Chamorro (1936), Dworzak and Podleschka (1936), Bazy and Jourdan (1938), Lane and Markee (1941), May (1944), and Ward, Gardner and Newton (1953).

Histological observations have been widely used to determine the behaviour of various elements in ovarian grafts including follicles, corpora lutea and interstitial cells, and in studying the host reaction to homografts and heterografts.

Autotransplants

As we have seen already ovarian autotransplants survive and regenerate under appropriate conditions. There has been some discussion of the origin of the various elements in an ovarian graft following regeneration, but according to Butcher (1932) ova can arise from the germinal epithelium.

There is convincing evidence that Halsted's principle (p. 25) holds good for ovarian transplants, in the sense that they are likely to regress unless the recipient is either oophorectomized or receives injections of gonadotrophin (*see* e.g. Ingram and Krohn, 1956)

Homotransplants

Ovarian homotransplants between unrelated animals, other than those located in special sites such as the anterior chamber of the eye, resemble homografts of other tissues in that, as a general rule, they are eventually destroyed (*see* e.g Hannan, 1929, and Miller, 1934, in addition to the papers cited previously). It is almost impossible however, after reading critically the early liter-

ature on the subject to escape the conclusion that ovarian homotransplants may survive for weeks or even months in various different species and at least as far as the rat is concerned this has been established beyond question by the comparatively recent work of Harris and Eakin (1949) Ingram and Krohn (1954 1956) Krohn (1955), Billingham and Parkes (1955) and Parkes (1956b) It is apparent therefore that ovarian homotransplants may survive very much longer than skin homografts exchanged between animals showing a similar degree of genetic diversity The reason for this is by no means clear Billingham and Parkes (1955) found that as a general rule the immunity evoked by a skin homograft was effective against a graft of ovary from the same donor but occasionally ovarian grafts became established and functioned in animals which had rejected a previous skin graft from the same donor and when ovary and skin were transplanted simultaneously ovarian tissue was often present when the experiment was terminated 77 days later whereas the skin grafts had long since broken down

Harris and Eakin (1949) have suggested that the fate of an ovarian graft depends on the degree of hormonal stimulation to which it is exposed as well as on genetic factors and this indeed appears to be the case but virtually nothing is known of the mechanism involved It is of interest in this connexion that ovarian homografts may survive and function in male hosts as has been shown by many observers including Tamura (1927 Guyenot Bartschi and Ponse 1932) and Castillo (1932) Breward and Zuckerman (1949) concluded from experiments in rats that there was an organ specific component in the immunity to ovarian homografts but subsequently Mandl and Zuckerman (1951) withdrew this hypothesis

Homografts of foetal and immature ovaries have been studied by many observers including Kross (1925) May (1940) and Dunham Watts and Adair (1941) It has been suggested that such grafts survive more readily than homografts of adult ovary but decisive evidence on this point is lacking Dunham *et al* and May placed their grafts in the anterior chamber of the recipient's eye and as we have seen (p 56) this fact may have facilitated graft survival Other observers who have studied the behaviour of ovarian grafts in the anterior chamber of the eye include Schochet (1929) Goodman (1934) Payne and Meyer (1942) and Ward Newton and Gardner (1951) Intracerebral ovarian grafts have been studied by Athias and Guimaraes (1933)

Heterotransplants

There have been a number of reports of long survival of ovarian heterografts Manclaire and Lachowsky (1922) reported that ovarian grafts from a woman to the peritoneal cavities of two rabbits were still present four months later and on histological examination contained corpora lutea and numerous interstitial cells Subsequently Thorek (1930) reported that a human ovary transplanted to a monkey was still present 11 months later and contained atretic follicles Thorek considered that the graft produced a certain amount of oestrogen, but his evidence on this point is unconvincing and Hamman (1931) in a similar experiment obtained no evidence of function Pincus (1931) and more recently Sanders (1946) have recorded long survival of ovarian heterografts exchanged between rats and mice but their transplants were located in the ovarian capsules of the hosts and it seems likely that the results were due to regeneration of host ovarian tissue This interpretation would seem to gain some support from the observation of Turner (1937) that intra-ocular heterografts of mouse ovary in rats are rapidly destroyed

Storage of Ovarian Tissue

A great deal of work has been done on the storage of ovarian tissue notably by

Lipschutz and his colleagues (Lipshutz and Uprus, 1927; Lipshutz and Vesnjakov, 1927, 1928; Lipschutz, 1928a, b, 1929; Lipshutz and Kallas, 1930), and Parkes and Smith (Smith and Parkes, 1951, 1954; Parkes and Smith, 1953). This has been discussed in Chapter 9.

Clinical Observations

Autotransplants

Morris (1895) transplanted a small piece of ovary to the stump of one tube after removing both tubes and ovaries for bilateral salpingo-oophoritis. A month later the patient became pregnant, but she aborted at about three months. Frank (1898) reported three similar cases in which free ovarian transplants were placed either in the stump of a Fallopian tube or in the uterus after bilateral salpingo-oophorectomy. Menstruation was re-established in all three patients, and one of them became pregnant but subsequently aborted Similar operations were performed by Monprofit (1901), Pankow (1907), and Casalis (1909).

Dudley (1899, 1901) transplanted the ovary with its blood supply intact to the cavity of the uterus after removing both tubes and the opposite ovary. Menstruation was restored, and in one case a period of amenorrhoea ensued which may possibly have been due to pregnancy.

The transplants we have been considering may reasonably be described as orthotopic (p. 5). Mauclaire (1900) appears to have been the first to use heterotopic ovarian transplants He placed pieces of ovary in the subcutaneous tissue and reported that menstruation was subsequently re-established. Later (Mauclaire, 1908, 1909) he attepted heterotopic transplantation by vascular anastomosis in two patients, anastomosing the inferior epigastric artery to the vein of the transplant. Mauclaire also used orthotopic transplants and reported that pregnancy occurred in some cases.

Cramer (1906, 1919) at first used free orthotopic transplants, but later reported that heterotopic transplants gave better results as far as the endocrinological status of the patient was concerned.

Martin (1903, 1908, 1911, 1915, 1917, 1922) made an extensive study of free orthotopic transplantation following bilateral salpingo-oophorectomy for tubo-ovarian suppuration, and performed the useful service of publishing a series of reviews of the literature on ovarian transplantation in general.

Estes (1909, 1922, 1924a, b, 1925, 1932) devised a new technique for pedicle transplantation in which a portion of one ovary, with its vascular connexion to the uterus intact, is placed in the stump of one tube after removing both tubes and the opposite ovary. Estes and Heitmayer (1934) reviewed 30 patients treated in this way and reported that 4 of them had become pregnant. In two cases the pregnancy continued normally to term, and one of the patients subsequently became pregnant again three times.

Tuffier (1911, 1913b, 1915) made subcutaneous transplants to the abdominal wall in a large number of patients following salpingo-oophorectomy, but later he used mainly pedicle transplants to the cavity of the uterus (Tuffier and Bour, 1924, 1925).

Heterotopic ovarian autotransplants have been used by many other surgeons including Davidson (1912), Whitehouse (1913), Chalfant (1915), Norton (1915), Phillips (1917), Nattrass (1915, 1923), Graves (1917), Cotte and his colleagues (Cotte and Colson, 1933; Cotte, 1936a, b, c; Cotte and Mathieu, 1941), Unterberger (1918a, b), Blair-Bell (1920, 1925), Bainbridge (1923b, 1932), Norris and Behney (1929), Douay (1932, 1939), Nard (1933), Lapeyre (1937), Cheval and Mayer (Mayer, 1934; Cheval and Mayer, 1933; Cheval, 1934, 1939, 1946), Mandelstamm (1935), Knudtzon (1937), Ezes and Laffargue (1938), Counseller and Wrork (1940), Kuhn (1943), and Buxton and Wong (1950). The

formation of cysts in such grafts has been reported by Lack (1923) and King (1932).

For the most part heterotopic transplants have been placed in the subcutaneous tissue of the abdominal wall, in the sheath of the rectus muscle, or in muscle tissue, but good results were reported by Macclure (1917, 1922) and Cotte with grafts placed in the omentum, and by Douay, Nard, Lapeyre and Knudtzon with grafts placed in the labium majus.

In a case reported by Nattrass, Caesarean section was combined with bilateral oophorectomy and autotransplantation of the ovaries to the rectus sheath and to the subcutaneous tissue of the abdominal wall. Lactation became established, and when it ceased regular menstruation was resumed.

Unterberger stated that there were only two indications for ovarian transplantation, namely, bilateral benign ovarian tumours which cannot be resected without removing both ovaries completely, and severe chronic adnexal infection which does not respond to conservative treatment. He reported that the chances of establishing regular menstruation in women of appropriate age in whom the uterus was left *in situ* were excellent if healthy pieces of ovarian tissue were cut up into small fragments and transplanted to the rectus sheath.

Blair Bell by 1920 was able to report 98 cases in which he had performed ovarian transplantation, and by 1931 (*see* discussion to Cheval, 1931) the number of his cases had risen to 218. He emphasized very strongly that whenever possible the ovary should be preserved in its natural situation, but if this appeared to be impossible or inadvisable, as in many patients with severe tubo-ovarian suppuration or bilateral benign ovarian tumours, he recommended and himself performed ovarian transplantation.

Cheval and Mayer reported that when the uterus was preserved menstruation was re-established in about 80 per cent of cases by means of intramuscular or subcutaneous ovarian autotransplants. When the uterus was removed ovarian transplantation was still beneficial in mitigating menopausal symptoms, but they considered that to obtain the best results strips of endometrium should be transplanted at the same time. They performed experiments in dogs which appeared to support this contention, but it has not been confirmed by subsequent work.

Mandelstamm carried out ovarian transplantation after performing a radical operation for carcinoma of the cervix, but the obvious danger of this procedure has deterred others from following his example.

Heiberg (1937) made repeated estimations of urinary gonadotrophin in five of Knudtzon's (1937) patients. He observed no change for several weeks after the transplantation and assumed from this that oestrogen was being absorbed from the graft. Thereafter the level of urinary gonadotrophin rose, suggesting that the store of oestrogen in the graft was exhausted and that the graft had not yet begun to function efficiently. After a month or two the level returned to normal, suggesting that the graft was actively functioning. Finally, some months later the level again rose, and Heiberg assumed from this that the graft had reached its climacteric.

Counseller and Wrork reported that ovarian autotransplantation was performed on 136 patients at the Mayo Clinic from 1915 to 1940. They stated that the indication for this procedure is a disease of the ovaries which cannot be eradicated while ovarian tissue remains in the pelvis, and under this heading they included cases of endometriosis. They found that the best results were obtained if healthy pieces of ovary were shaved into thin slices and grafted to the sheath of the rectus muscle. They claimed that the grafts held the symptoms of the menopause in abeyance, prevented atrophy of the genitalia, maintained certain feminine attributes, and allowed a

milder, more natural menopause, instead of an abrupt, severe artificial menopause."

Kuhn reported 11 cases in which a piece of ovary had been transplanted to the rectus sheath following bilateral oophorectomy without removal of the uterus. Menstruation was re-established in every case, and some of the patients were still having regular periods 7-8 years after the operation.

Buxton and Wong reviewed 41 cases in which transplantation of ovary to the rectus sheath was performed in association with oophorectomy and other procedures in patients with chronic pelvic inflammation, benign ovarian cysts, endometriosis, and ectopic gestation. They pointed out that this procedure avoids the dangers of persistent infection and cyst formation which obtain if pieces of ovary are left in the peritoneal cavity. Menstruation was re-established in 4 out of 5 patients in whom the uterus had been retained, and vaginal smears and estimations of urinary oestrogens provided evidence of graft function in a number of other patients who had been subjected to hysterectomy. Buxton and Wong concluded that ovarian autotransplantation was of definite value, and deprecated the fact that the operation was not more widely practised.

Autotransplantation of the corpus luteum was performed by De Lee (1916) in two patients with ovarian cysts who were 8 months pregnant, but in neither case was the procedure successful.

Orthotopic transplantation of the ovary has not been used as extensively as heterotopic transplantation, but, in addition to the cases cited previously, instances of pregnancy following this procedure* have been reported by Storer (1914), Bainbridge (1923a), Foster (1925, 1930), Pavlik (1928), Risley (1935), Panis (1937), and Sturgis (1939). Both free and pedicle transplants have been used.

*Combined with removal of all other ovarian tissue.

Homotransplants

Morris (1895) appears to have been the first to report on the clinical use of an ovarian homograft. The recipient was a girl aged 20 suffering from amenorrhoea associated with an infantile uterus, and the donor was a woman of 30 The graft was placed in the fundus of the uterus. Menstruation started two months after the operation, but later ceased, and Morris (1901) concluded from this and other cases that ovarian homotransplants undergo fatty degeneration and cease to be functionally effective within a year. Later however (Morris, 1906a, b) he reported a case in which pregnancy culminating in the delivery of a living child occurred four years after ovarian homotransplantation, and he attributed this to prolonged survival of the transplant in consequence of fortuitous compatibility of donor and host.

Many further instances of ovarian homotransplantation have been reported. In some re-establishment of menstruation or other evidence of endocrine activity in the graft has been observed following either orthotopic or heterotopic transplantation (*see* e g Cramer, 1906; Engel, 1912, 1924; Hooper, 1912; Lydston, 1916; Bainbridge, 1923b; Loesser, 1926; Gambarow, 1929; Solomons, 1931; Bennett, 1940, Bennett and Russell, 1941), and, in a few of the cases in which the transplants were orthotopic, pregnancy has occurred and has been attributed to fertilization of an ovum liberated by the transplant (Lucas-Championnière, 1907; Sippel, 1924, 1926, 1928). *Much of this evidence* is unconvincing, however, because the possibility of reactivation or regeneration of host ovarian tissue can rarely be excluded, and in many other cases ovarian homotransplants have been frankly unsuccessful (*see* e.g. Quénu and Sauvé, 1909; Martin, 1911; Tuffier, 1911, 1913, 1915; Unterberger, 1918b).

In recent years interest in the subject of ovarian homotrans[illegible]

tirely lapsed despite the enthusiasm of a few staunch advocates such as Bennett. This is perhaps just as well in view of the somewhat irresponsible way in which the whole or part of a healthy ovary was sometimes removed for use as a homotransplant from a young donor undergoing a relatively minor pelvic operation such as a Gilliam suspension of the uterus. This criticism does not apply to Bennett, who for the most part interchanged ovarian grafts between women suffering from contrasting types of ovarian dysfunction. Various explanations other than graft survival may be suggested to account for his results, and his procedure may be criticised on theoretical grounds, but it is at any rate relatively innocuous.

Heterotransplants

Heterotransplantation of monkey ovary to women has been reported by Latis Bey (1927, 1929, 1931), Pende (1928) and Rubinstein (1931). Latis Bey's patient began to menstruate regularly after the operation and was still doing so two years later, but it seems not unlikely that the patient's own ovaries began to function again. There is in fact no convincing evidence that heterotransplants function in any biological sense, though they may perhaps constitute a depot of oestrogen and thus act for a short time in the same way as an implant of this hormone.

TRANSPLANTATION OF OVARY IN CLINICAL SURGERY. PRESENT STATUS AND FUTURE PROSPECTS

Autotransplants

When both ovaries have to be removed from a young or middle-aged woman there is seldom much healthy ovarian tissue available for transplantation, but fortunately the endocrinological consequences of the operation can largely be controlled by hormone therapy. As a result the possibility of ovarian autotransplantation is nowadays rarely given serious consideration. In the occasional case where transplantation is possible, however, it has much to commend it. Heterotopic transplantation of small pieces of ovary to the rectus sheath is simple and safe, and appears to be highly effective. Moreover, if transplantation proves unsuccessful, orthodox forms of hormone therapy can be instituted.

Orthotopic transplantation raises other issues. Whatever form of transplantation is contemplated the danger of transmitting infection or neoplastic disease must be carefully weighed, but it seems desirable to apply even more stringent criteria when the whole or part of the ovary is to be replaced in the peritoneal cavity than when it is to be transplanted, for example, to the abdominal wall. In consequence free orthotopic autotransplantation will rarely be justified, except when an unnecessarily radical ablative operation has been carried out, and part at least of one ovary could and should have been retained. When, however, a Fallopian tube is resected without removing the corresponding ovary, and there is doubt about the efficacy of the opposite tube and ovary, the possibility of pedicle transplantation of the sound ovary to the stump of the tube or the cavity of the uterus should be considered. This procedure is not often carried out, but there is no doubt that it may on occasion permit pregnancy to occur in a patient who would otherwise be sterile.

Homotransplants

There is clearly no justification at the present time for using ovarian homotransplants to deal with the endocrinological consequences of loss of the ovaries, because the chances of success are small and hormone implantation provides a satisfactory method of treatment.

Is homotransplantation justifiable in the hope of making it possible for pregnancy to occur? The orthodox answer is no, but as we have seen the behaviour of ovarian homotransplants both in man and in experimental

animals differs considerably, for example, from that of skin homografts, and it seems likely that in some at least of the reported cases in which pregnancy has followed ovarian homotransplantation the ovum which was fertilized came from the transplant. It must be recognized however that the chances of success are small, and also that, if the operation is successful, it will raise highly controversial ethical and legal questions. It is desirable that these questions should be faced because, if a general solution to the homograft problem is found, ovarian homotransplantation seems likely to be requested by a considerable number of patients.

If ovarian transplants are used they should consist of fresh tissue. It is true that ovarian tissue can remain viable when preserved in the frozen state, but to use a stored homotransplant would reduce still further the small chance that the operation will be successful.

Heterotransplants

In the author's view ovarian heterotransplants have no place in present day surgery.

TRANSPLANTATION OF TESTIS

HISTORY

Animal Experiments

Early Work

John Hunter (1771) stated* that he had "frequently taken out the testis of a cock and replaced it in his belly, where it has adhered and has been nourished," and also that he had "put the testis of a cock into the belly of a hen with the same effect." Unfortunately however, no detailed account of these experiments exists,† and priority in the matter of experimental transplantation of the testis is usually accorded to Berthold (1849), who removed the testes of cocks, cut them up and replaced the pieces in the peritoneal cavity, and observed that normal sexual development occurred.

In discussing subsequent developments it will be convenient to consider autotransplants, homotransplants and heterotransplants separately.

Autotransplants

Free autotransplants of testis have not been studied to anything like the same extent as homotransplants and heterotransplants. What is even more surprising is the high proportion of unsuccessful experiments which have been reported (*see* e.g. Loeb, 1918c; Ocaranza, 1922; Sechi, 1923; Aron, 1929). Today it is generally regarded as essential to establish a technique of transplantation which is consistently successful with autotransplants before embarking on a study of homotransplants or heterotransplants, but this consideration apparently carried little weight with many of the earlier workers in this field.

Among the successful experiments those of Lundy (1925) are of special interest. He observed survival of both interstitial cells and seminiferous epithelium in autotransplants of fresh testicular tissue, whereas transplants of testicular tissue which had been stored at ice-box temperature for 24 hours or longer were uniformly unsuccessful.

Aron (1929), who made an extensive study of autotransplants (and also homotransplants) to the tunica vaginalis and peritoneal cavity in mice, rats and guinea pigs,

*See Forbes (1947) for a scholarly discussion of Hunter's experiments on this subject. A useful bibliography of testis transplantation has been published by Krohn (1955e). Moore (1926) has reviewed the early history of the subject.

†Forbes (1947) suggests that Hunter's notes on testis transplantation were among the papers destroyed after his death by Sir Everard Home.

concluded that the failure of autotransplants was due primarily to the sensitivity of testicular tissue to ischaemia This is certainly a factor and suffices to explain why Aron and others were unsuccessful with free grafts of whole testis but it is difficult to see why in so many experiments small transplants were equally unsuccessful

The failure of free whole testis transplants was exploited by Moore (1930) in an attempt to determine whether hormone was liberated in significant amount by regressing testicular transplants He found however no evidence that this occurred in *either guinea pigs or rats*

In recent years Williams (1949 1950) has made important observations with testicular autotransplants in modified Sandison Clark ear chambers in rabbits Some of the transplants contained interstitial cells but no seminiferous tubules some consisted almost exclusively of seminiferous tubules and some contained both interstitial cells and tubules Williams observed that interstitial cells developed from cells which were indistinguishable from fibroblasts and seemed to have a life span of about nine months When interstitial cells were present Sertoli cells persisted and produced what was interpreted as a secretion Spermatogenic cells persisted but after the first few months spermatogenesis did not proceed beyond the stage of secondary spermatocytes

Pedicle transplants have been used to study the effect of local temperature on the testis and to determine whether endogenous androgens are inactivated by passage through the liver

It has long been known that in cryptorchidism spermatogenesis does not occur in the misplaced testis and in 1922 Crew suggested that this is because when the testis lies outside the scrotum which is efficiently air-cooled the local temperature is too high Moore (1922 1924a b c) investigated the matter experimentally in guinea pigs rabbits and other animals He found that if the testis was transplanted to the peritoneal cavity with its spermatic cord intact the germinal epithelium underwent gross degenerative changes within a few days and after 20 days little remained of the seminiferous tubules except the supporting reticulum the Sertoli cells lying on the basement membrane and a few spermatogonia Moore showed also that spermatogenesis occurred in free transplants of testis made to the scrotal wall though it did not normally occur with free transplants in other sites

The claim that these phenomena can be explained by *the relatively low temperature* in the scrotum rests on three main grounds In the first place direct measurement of the scrotal temperature shows that it is in fact lower than the temperature in the peritoneal cavity The difference varies with the species of animal and the temperature of the environment in a white rat when the room temperature was 16°C the difference was 8°C (Moore and Quick 1924) Secondly if the scrotum of a ram is enclosed in woollen material with a waterproof covering the testes are found to be devoid of spermatozoa after 80 days and show gross tubular degeneration (Moore and Oslund 1924) Similar changes develop in about 10 days if the temperature of the scrotum of a guinea pig is artificially raised about 7°C by the application of hot pads for as short a period as 15 minutes (Moore 1924b) Thirdly if both testes are displaced into the peritoneal cavity and the body surface over the site of one of them is artificially cooled this testis retains its normal structure whereas the other which is not cooled shows typical degenerative changes (*Fukui* 1923a b c)

The possible effect of the liver on androgenic hormones was investigated by Krichesky Benjamin and Slater (1943) The prostate gland was removed from young male rabbits and part of it was transplanted autologously to the anterior chamber of the

eye. Three weeks later the testes were brought up into the peritoneal cavity and sutured to the jejunum. Finally, when sufficient time had elapsed for a new blood supply to become established, the spermatic cords were severed, with the object of diverting the whole of the venous drainage of the transplant to the portal system. As soon as this was done the prostatic grafts, which had hitherto remained healthy, began to regress. This was attributed to destruction by the liver of androgen secreted by the testicular transplants, and the matter appeared to be placed beyond doubt when it was shown further that the regression of the prostatic transplants could be arrested by suturing the testicular transplants to the abdominal wall and thus re-establishing a connexion with the systemic venous system.

Homotransplants*

Many investigators claim to have demonstrated endocrinological effects mediated by testicular homotransplants.

Wheelon and Shipley (1915) observed that the vasomotor response to nicotine in dogs, which was greatly reduced following castration, was partly restored 10 days after intramuscular transplantation of slices of testicular tissue, but for the most part these claims have been based on increased activity, restoration of sexual function, and other evidence of rejuvenation in senile animals including rats (Romeis, 1921), guinea pigs (Harms, 1920), and rams and goats (Retterer, 1919a, b, 1929; Boukalik and Hoskins, 1927); increased spontaneous activity in castrated male rats (Richter and Wislocki, 1928); or masculinization in spayed female rats (Moore, 1919) and guinea pigs (Moore, 1921).

Other experiments may be cited in which no evidence of function was obtained (e.g. Bolognesi, 1921; Hoskins, 1925), but it seems nevertheless to have been definitely established that testicular homotransplants may produce the effects described. As a general rule* however, as Retterer (1929) pointed out, they do so only for a limited time.

It has been reported further by Voronoff (1927) and others (e.g. Male, 1931; Runge, 1943) that testicular homotransplants may be used to produce effects of economic importance in domestic animals, including increased production of wool in sheep, prolonged restoration of potency in dogs, stallions and bulls which have ceased to be useful stud animals on account of their age, and even the transmission to the offspring of the host of acquired characters of the donor. The French authorities in Algeria provided facilities for many of Voronoff's experiments, and in 1928 a British delegation visited Algeria and prepared a report on this work for the Ministry of Agriculture and Fisheries and the Board of Agriculture for Scotland. Their verdict (Marshall, Crew, Walton and Miller, 1928) was essentially one of not-proven; i.e. they did not state that Voronoff's claims were false but criticised as inadequate the evidence on which they were based. It seems clear, however, from the subsequent careful investigations of Velu and Balozet (1928, 1929a, b, 1931), Kucera (1929), Quinlan and Marais (1931), Gunn and Seddon (1931), and others (*see* Velu, 1931, for review) that Voronoff's conclusions must be rejected.

Histological observations in various species, despite a few claims to the contrary, have established that, as a general rule, testicular homografts, like homografts of other

*Observations in poultry, subsequent to those of John Hunter and Berthold, have not been included in this review but are of considerable biological interest (*see* e.g. Pézard, Sand and Caridroit, 1921, Domm, 1924, 1930, Greenwood, 1924a, b Caridroit, 1925, 1928, Greenwood and Blyth, 1930) They have largely been concerned with the endocrinological effect of testicular homotransplants in female hosts. Interpretation of the experimental findings has proved difficult, however because a testis like gonad may develop in an oophorectomized hen

*There are of course exceptions, as for example when the donor and host are members of an inbred strain.

cryptorchid testis to a previously castrated monkey, and observed what he described as marked erotization. He also claims to have successfully transplanted testes between various species of higher apes, but to the author these experiments are unconvincing.

Crisler (1929) transplanted testes from white mice to the tunica vaginalis of hemi-castrate rats. The grafts were removed for histological examination after about six months. All showed marked degenerative changes, though seminiferous tubules could sometimes be identified. Browman (1937), in a rather more extensive series of experiments, found that heterografts from rats to mice and *vice versa* could be successfully transplanted back to the donor after four days in the host. Heterografts which were left in the host were said to remain in a state of fair preservation for periods up to two months, but no evidence of endocrine activity was obtained.

Storage of Testicular Tissue

Deanesly (1951), working in Parkes' laboratory, found that testes from prepubertal rats retained their capacity to grow and differentiate as intrastrain grafts after soaking in 15% glycerol-saline and freezing to −79° C. (*see* Chapter 9).

Clinical Observations

Autotransplants

It has been established that extra-scrotal testes* up to the age of puberty have the same structure as scrotal testes (*see* Wangensteen, 1927, 1932), but thereafter scrotal testes increase in size and show normal spermatogenesis whereas in extra-scrotal testes spermatogenesis is seriously impaired. As we have seen (p. 504), there is good evidence that this difference is due to the relatively low temperature of the scrotal testis. The endocrine function of the extra-scrotal testis is often said to be normal, or nearly so, but there is evidence (Engberg, 1949) that it too may be significantly diminished. In addition, when the testis lies outside the scrotum, there is often an associated inguinal hernia, the risk of injury is increased unless the testis happens to lie within the abdomen, and there is evidence that the risk of malignant change is somewhat higher than normal. John Hunter, who published the first adequate description of the normal descent of the testis, believed that failure to reach the scrotum was due to some intrinsic abnormality of the testis itself, but it has since been established that the development of the testis may be improved if it can be transplanted to the scrotum while keeping the spermatic cord intact (*see* Wangensteen, 1927, for review). This operation, which is known as orchidopexy, was attempted without much success by Rosenmerkel (1820), Chelius (1837), Partridge (1858) and Adams (1871), but was subsequently shown to be feasible by Schüller (1881) and Bevan (1899, 1903). Since then various modifications have been introduced, but it will be more convenient to consider these later (p. 509).

It is sometimes impossible to bring the testis into the scrotum, and in four such cases Bailey (1927) excised the testis and replaced it as a free graft in the rectus sheath. He did not report the results of this procedure, however, and it seems extremely unlikely that it was of any benefit to the patients.

Kearns (1941) reported an interesting, and apparently successful, instance of autotransplantation of the testis. The patient was a man aged 23, and both his testes and part of the skin of the scrotum were avulsed in an accident. The testes were placed in a refrigerator, and next morning slices 2 mm. thick were transplanted into the wound and covered with flaps formed from the remaining scrotal skin. Six weeks after the operation the prostate was scarcely palpable, but after six months it was of normal

*This term is used to include testes situated somewhere along the normal line of descent (*undescended testes*) and also testes in other situations (*ectopic testes*)

size and prostatic massage yielded secretion of normal lecithin content Similar findings were obtained 26 months after transplantation and at the same time androgen was shown to be present in the urine It would seem therefore that the grafts were endocrinologically active

Homotransplants

Hammond and Sutton (1912) according to Lespinasse (1913) performed homotransplantation of the testis by making end to-end anastomoses of the spermatic artery and vein This difficult technical feat was apparently accomplished successfully but despite this the transplant atrophied The patient had one healthy testis of his own however and this may have contributed to the failure of the transplant

Lespinasse (1913) himself made free transplants consisting of thin slices of testis about 1 mm thick to the rectus sheath and scrotum of a man aged 38 who had lost both his own testes He claimed that potency was restored and was still present two years later

Further claims of successful homotransplants were put forward by Morris (1914 1916) Lydston (1914a b 1916 1918a b 1921) Lichtenstern (1916 1920 1921) Kreuter (1919) Mariotti (1919 1928) Stanley and Kelker (1920) McKenna (1921) *Gregory (1922) Muhsam (1922) Voronoff* (1922) Walker (1924) and Bailey (1927) The patients were suffering from a variety of conditions including traumatic loss of both testes atrophy of the testes due to mumps and other causes genital infantilism senile atrophy of the testes homosexual tendencies and schizophrenia

Most of the transplants were obtained from young men killed in accidents Stanley who was medical officer at San Quentin *prison obtained testes from executed criminals* McKenna and Walker used undescended testes removed at operation Lydston stored some of his transplants for up to 36 hours in saline on ice but for the most part the grafts were used fresh Sometimes the testes were transplanted whole but more commonly they were sliced or minced Various sites were employed including the rectus sheath (Lichtenstern Morris) the tunica vaginalis (Voronoff Lydston Walker) and the subcutaneous tissue (Stanley)

The benefits reported ranged from rather vague improvement in the patient s general mental and physical state to complete restoration of the secondary sexual characters and of potency

Many other cases were reported (Enderlen 1921 Stabel 1921 Forster 1922 Kreuter 1922a b Brandt and Lieschied 1923 Sand 1923) in which testicular homotransplantation had either a very transient effect or no effect at all and in some of these it was found on histological examination that the grafts had been completely destroyed within a few months It seems likely therefore that in the apparently successful cases the benefits claimed were due either to psychological factors or as Stanley and Kelker (1920) first suggested to absorption of androgen from a graft in process of destruction

Heterotransplants

Stanley and Kelker (1920) made testicular heterotransplants from rams to five patients *They did not report the cases in detail* but the results appear to have been discouraging except in one elderly patient who was said to have benefited from the operation despite the fact that much of the graft sloughed Subsequently however Stanley (1921 1922 1931) reported that improvement in general health occurred in a large number of patients following subcutaneous injection of minced testis from goats rams boars and deer Further cases showing improvement *in general health and sometimes recovery* of potency following heterotransplantation of ram or goat testis were reported by Lichtenstern (1921) Hunt (1922) and Lipschutz and Krause (1924) It is noteworthy that in

many of these cases beneficial results were reported to occur despite the fact that the grafts sloughed.

Voronoff initiated the use of heterografts of testis from monkeys, and he, with his pupils and colleagues, claimed that it was possible by using such grafts to improve the general health and mental state of many elderly patients, and in some cases to restore potency (Voronoff, 1922a, b, c, 1923, 1925; Dartiques, 1923; Retterer and Voronoff, 1923a, b; Thorek, 1924; Retterer, 1926, 1927a, b; Voronoff and Alexandrescu, 1930; Retterer and Alexandrescu, 1934). In consequence "rejuvenation by grafting monkey glands" captured the public interest, and was widely practised in a number of clinics on the continent and also in America.

It now seems clear, however, that any benefit which occurred must have been either psychogenic or the result of absorption of hormone liberated by grafts in the process of destruction.

TRANSPLANTATION OF TESTIS IN PRESENT DAY SURGERY

There are definite indications for supplying a patient with androgens—though the propriety of attempting to rejuvenate old men in this way is, to say the least, open to question—but this is now achieved by injecting testosterone, or by administering one of its derivatives such as methyltestosterone by mouth. In consequence homotransplantation and heterotransplantation of the testis have no place in present day surgery, and we need therefore consider only autotransplantation.

Free Autotransplants

The opportunity for performing free autotransplantation of the testis seldom arises, but the procedure should always be attempted in cases of accidental castration if a reasonable amount of uninjured and uncontaminated testicular tissue is available. It would seem best to use thin slices of tissue, and to place these in the sheath of the rectus abdominis muscle.

Orchidopexy

Orchidopexy, i.e. pedicle transplantation of an extra-scrotal testis to the scrotum, is a common surgical procedure. In the author's view it is indicated in all boys in whom the testis is unlikely to reach the scrotum unaided but can be brought there by operation.*

The operation is usually performed at the age of 10-12 years, but may with advantage be undertaken earlier if the testis is ectopic as distinct from undescended or if there is a troublesome associated hernia. The chances of success are greatly lessened if the operation is delayed until after puberty, though there is some evidence that it may still be of value in a proportion of patients (Pace, 1935; Rea, 1942).

The first step is to mobilize the testis sufficiently for it to be brought down into the scrotum without undue tension on the structures of the cord. With an inguinal ectopic testis—situated in what Denis Browne calls the superficial inguinal pouch—this is easy; with testes at the external ring or in the inguinal canal it may be difficult; and with intra-abdominal testes it is usually impossible. The limiting factor, once the hernial sac has been dealt with, is the shortness of *the spermatic artery and vein,* and the manoeuvre described by Gross (1953) of dividing the internal oblique and transversus muscles lateral to the normal point of emergence of the cord greatly facilitates the dissection of these vessels

Once the testis has been mobilized it is

*Patients with retractile testes which can be brought to the scrotum by simple manipulation do not require any treatment In other cases some paediatricians advise a course of injections of gonadotrophin in preference to operation. The author, however, agrees with Denis Browne (1938) that this form of treatment will not bring down any testes that would not have descended unaided to the scrotum sooner or later, though it may hasten the descent.



Fig. [illegible] Keetley's illustration of his method of orchidopexy (from the *Lancet* [illegible]). The original legend states that the operation had been performed two years previously and, being perfectly comfortable, the patient did not wish the testis to be separated from the thigh. (Reproduced by courtesy of the publishers of the *Lancet*.)

placed in a bed prepared for it in the scrotum by blunt dissection, and steps must then be taken to ensure that it remains in its new situation. Bevan (1899, 1903, 1929) used a purse-string suture at the neck of the scrotum for this purpose. Keetley (1894, 1900) brought the testis out through an incision in the scrotum and attached it to the fascia lata after making a further incision in the thigh. He then joined the skin of the scrotum to that of the thigh at the margins of the two incisions. At a second operation he separated the scrotum from the thigh, returned the testis to the scrotum and closed the incisions (Fig. 157). This operation was subsequently re-discovered by Torek (1909) and modified by Wangensteen (1932) and other surgeons.

The Keetley-Torek procedure is open to the criticism that it is either unnecessary or, if the testis cannot be retained in the scrotum in some less forcible way, the spermatic vessels will be subjected to such tension that they will go into spasm and the vascular supply of the transplant will be imperilled. The author therefore prefers the simpler procedure illustrated in Figure 158, in which a stitch is passed through the tissue at the lower pole of the testis, brought out through the scrotum, and connected to a piece of elastic adhesive strapping attached to the thigh. Only slight tension is permitted, and the stitch is removed after a few days.

What are the chances that a patient will be fertile after bilateral orchidopexy? This question has been much discussed (*see* e.g. MacCollum, 1935; Wangensteen, 1935; Hansen, 1949; Gross, 1953) but must be regarded as still *sub judice*. It seems likely, however, that whereas surgeons have often taken an over-optimistic view of the situation, many endocrinologists have been unduly pessimistic.

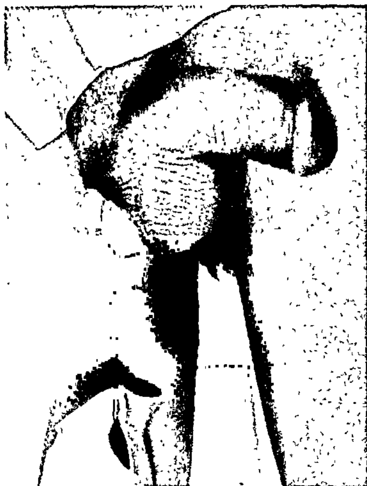


Fig 158 A simple method of retaining the testis in the scrotum after orchidopexy A suture is passed into the scrotum, through the remnants of the gubernaculum at the lower pole of the testis, and out again. It is tied over a small roll of gauze and the ends are attached to a piece of elastic adhesive strapping which is fixed to the thigh. Only very slight tension is employed.

CHAPTER 25

Transplantation of the Kidney

BASIC PRINCIPLES GOVERNING THE TRANSPLANTATION OF WHOLE ORGANS

Successful autotransplantation of a whole organ such as the kidney by vascular anastomosis is contingent on three factors the development of a satisfactory operative technique the avoidance of irreversible ischaemic damage to the organ during the procedure, and the capacity of the organ to function when deprived of its nerve supply

The conditions for successful autotransplantation are also necessary, though they are not of course sufficient, for permanently successful homotransplantation, which requires in addition some means of avoiding or overcoming the homograft reaction, and, if the potential clinical value of the procedure is to be fully realized, the development of satisfactory methods for storing organs

It will be helpful to keep these principles in mind when considering the special case of transplantation of the kidney

HISTORY OF RENAL TRANSPLANTATION

Animal Experiments from 1902 to 1939

Autotransplantation of the dog s kidney by anastomosis of the renal vessels to the great vessels in the neck was performed first by Ullmann (1902), and very soon afterwards by Carrel (1902) but the transplants either failed to function or functioned for a very short time In the same year that these experiments were reported von Decastello (1902) published an account of homotransplantation of the kidney, but again the experiment must be reckoned a failure as the transplant was found to be necrotic after 48 hours

The development of satisfactory methods of vascular anastomosis by Carrel and others (Chapter 21) stimulated further research in organ transplantation, and during the next twenty years autotransplantation of the kidney was studied in dogs and less frequently in cats, by many investigators, including Carrel and Guthrie (Carrel and Guthrie 1905b, Carrel, 1908a 1909, 1910a, 1911), Capelle (1908), Zaaijer (1908), Villard and Tavenier (1910), Lobenhoffer (1913), Dederer (1918) and Lexer (1919) Three main techniques were used transplantation to the neck with anastomosis of the renal vessels to the divided carotid artery and jugular vein, transplantation to the groin with anastomosis to the femoral vessels and abdominal transplantation with re anastomosis of the renal vessels, or anastomosis of the vessels of the transplant to the splenic or iliac vessels, or to the aorta and vena cava As a rule cutaneous ureterostomy was established, but sometimes with abdominal transplants the ureter was re

paired by end-to end anastomosis or the portion of ureter attached to the transplant was connected to the bladder.

There were many failures, due partly to technical difficulties and partly to infection, but there were also many instances of survival for weeks or months. In one experiment reported by Carrel (1909, 1910b, 1911) a bitch lived for more than two years after transplantation of one kidney to the neck and removal of the other kidney. The animal gave birth to two litters of puppies during this period and died eventually of intestinal obstruction. Another dog in which Zaaijer (1908) transplanted one kidney to the inguinal region and anastomosed the renal vessels to the external iliac artery and vein survived for six years after removal of the other kidney.

While this work was going on there were in addition many studies of renal homotransplants. Floresco (1905) found that homotransplants in the neck functioned for periods ranging from two to nine days after which they were destroyed by infection and necrosis. Carrel and Guthrie had much the same experience and came to the conclusion that the neck was an unsatisfactory site for homotransplants. They obtained somewhat better results with intra-abdominal transplantation, using the patch method of vascular anastomosis (*see* Fig. 16), or alternatively the technique which they termed *transplantation in mass* (Carrel and Guthrie, 1906c; Carrel, 1906, 1908b), in which segments of donor aorta and inferior vena cava bearing the renal vessels were left attached to the transplant and were inserted into gaps made by transecting the aorta and inferior vena cava of the host. Carrel (1908b) reported one instance in which a cat survived 36 days after bilateral nephrectomy and intra-abdominal homotransplantation in mass, and stated that the transplant showed little change in structure at the end of this time, but the average period of survival was only about 16 days, and while some transplants appeared fairly normal on histological examination others showed changes resembling interstitial nephritis. Unger (1909, 1910), who performed many experiments in dogs and cats, obtained results no better than those of Carrel and reported that none of his animals survived more than 18 days after homotransplantation. Borst and Enderlen (1909b) also found that the recipients of kidney homotransplants died within 18 days, and reported that the transplants showed marked degenerative changes and necrosis. Ingebrigsten (1914) obtained survival up to 24 days, but found that the transplants showed lymphocytic infiltration and other inflammatory changes. Dederer (1920) reported that a kidney transplanted from one puppy to the neck of another member of the same litter continued to function until the host died 26 days later from distemper, but it seems possible that the close relationship of donor and host was the determining factor in this instance. No such explanation can be put forward however to account for the remarkable experiment reported by Avramovici (1924) in which a homotransplant maintained its normal structure, and the host survived, for 71 days.

The findings presented would appear to indicate that the behaviour of renal homotransplants and autotransplants is, with rare exceptions, fundamentally different; but this conclusion was by no means generally accepted by the earlier workers. Decisive evidence on this point was, however, soon provided by Williamson (1923, 1926) and Ibuka (1926a, b) working at the Mayo Foundation, and their papers merit detailed consideration.

Williamson (1923, 1926) found that an autologous kidney transplant in the neck, despite the fact that it was devoid of a nerve supply and was subjected to injuries and disturbances not encountered in the abdomen, would keep a dog alive for many months, and ceased to function only when hydronephrosis or infection supervened.

Homotransplants on the other hand whether in the abdomen or the neck functioned as a rule for only a few days, and cessation of function did not appear to be the result of infection but was associated with characteristic histological changes resembling those found in acute nephritis He concluded that the failure of homotransplants was due to a biologic incompatibility between the donor and recipient and that the variation in the length of survival of different transplants reflected a corresponding variation in the biological relationship of donor and host

Ibuka (1926a b) who likewise used dogs made a detailed study of the function of renal autotransplants and homotransplants He found that after autotransplantation to the neck albumen was usually present in the urine of the transplant for a short time but soon disappeared The concentration of chloride was at first considerably higher in the urine from the transplant than in the urine from the normal kidney but later the difference became less marked The capacity of the transplant to excrete nitrogenous substances phenolsulphonphthalein and sugar after the injection of phlorizin was usually less than that of the normal kidney but increased when the normal kidney was removed Homotransplants functioned on average for three days The volume of urine excreted by the transplant was greatest on the first day and decreased gradually or suddenly during the succeeding days until anuria set in The concentrations of nitrogenous constituents and chloride in the urine were low the first day increased to a maximum on the second or third day and then decreased either gradually or suddenly The excretion of nitrogenous constituents by the transplant was accompanied by a fall in the blood urea and to a less extent in the blood creatinine During the period of functional activity homotransplants responded like autotransplants to administration of water salt glucose, phlorizin, phenolsulphonphthalein and lithium carmine Albumen was present initially in the urine from the transplant it then usually disappeared for a time, but reappeared, together with red blood cells, pus cells and occasional casts, when the quantity of urine diminished Cessation of function was accompanied by swelling of the transplant and accumulation of blood stained fluid around it Histological examination of kidneys removed at this stage showed profound degeneration of glomerular and tubular elements with extensive interstitial infiltration of leukocytes and small round cells'

Wu and Mann (1934), also working at the Mayo Foundation, studied the histological changes in kidneys transplanted autologously and homologously to the neck in dogs by performing daily biopsies In contrast to the autotransplants, which showed slight and transient round cell infiltration for a day or two after operation, the homotransplants showed progressive round cell infiltration followed by degeneration and necrosis of the parenchyma Some specimens showed numerous polymorphs but this was thought to be due to ascending infection Thrombosis occurred in the vessels of some of the homotransplants but did not appear to be responsible for destruction of the transplant since it usually did not occur until necrosis was well advanced

Animal Experiments from 1947 Onwards

Interest in renal transplantation lapsed during the second world war but revived soon after the war ended

Experiments with autotransplants have been concerned mainly with the extent to which the behaviour of a transplanted kidney depends on the site in which it is located, the operative technique employed and the period for which the kidney is ischaemic Homotransplants have been studied to determine whether they conform

to the laws which govern the behaviour of free homotransplants of skin and other tissues, and, since this was found to be the case, to throw fresh light on the nature of the homograft reaction.

This work has been facilitated by the development of antibiotics, as a result of which the incidence of infection has been greatly reduced.

The Behaviour of Autotransplants

Parkinson and Woodworth (1947) transplanted kidneys autologously, and also homologously, to the neck in goats. They used vitallium tubes to anastomose the vessels to the carotid artery and jugular vein of the host, and established a cutaneous ureterostomy. Autotransplants developed such a good collateral circulation that they continued to function even when the main vessels thrombosed.

Hamburger (1947) demonstrated the value of heparin in diminishing the risk of thrombosis after renal transplantation. He also studied the effect of ischaemia by clamping the renal vessels without removing the kidney. He found that arterial occlusion for 15 minutes resulted in albuminuria, microscopic haematuria, and impaired urea concentration, which persisted unchanged for about 24 hours and then gradually diminished. In more than 50 per cent of dogs however renal function did not recover completely for more than a week. Venous occlusion, without interruption of the arterial supply, was even more serious, and if maintained for 15 minutes resulted in permanent impairment of function in more than 50 per cent of animals.

Hamburger also studied the effect of perfusing an excised kidney before replacing it in the host. He found that perfusion with Locke's solution was harmful, but perfusion with oxygenated heparinized blood was well tolerated.

Oudot (1918a), after studying kidneys transplanted to the neck in dogs, concluded that drainage of the urine by means of a cutaneous ureterostomy was unsatisfactory —a fact subsequently re-discovered by Dempster and other investigators. Oudot showed in particular that kidneys transplanted to the pelvis, with division and anastomosis of the vessels but without division of the ureter, functioned much better than kidneys transplanted to the neck.

Dempster (1950) studied the behaviour of kidneys transplanted to the neck in greyhounds, which he claimed were less subject to chronic infection of the urinary tract than most other dogs. He found that autotransplants usually began to secrete urine almost at once, survived indefinitely with little or no change in structure, and sufficed to keep the animal alive after removal of the other kidney. Occasionally the transplant failed to secrete or stopped secreting after a short time without any obvious cause, and he suggested subsequently (Dempster, 1953a, 1954) that this might be due to spasm of small vessels resulting from stimulation of renal nerves during the mobilization of the kidney.

Even in the most successful cases, however, a kidney transplanted to the neck did not function normally but produced urine of low specific gravity and was unable to retain sodium or chloride under conditions of depletion. Dempster (1950) considered various possible factors to account for this impaired function, including reduced renal blood flow, anoxia, denervation of the kidney, removal of the kidney from the neighbourhood of the adrenal gland, and lowered environmental temperature, and stated that in his view no single one of them was responsible, though some or all of them acting together might be

Subsequently Dempster and Joekes (1953) suggested that the functional impairment was due to interference with normal ureteric peristalsis caused by the cutaneous ureterostomy. Evidence in support of this hypothesis was obtained by Dempster, Joekes and

Oeconomos (1955), who showed that kidneys transplanted autologously to the pelvis, after an initial transient loss of tubular function, were able to excrete urine of normal specific gravity, concentrate urea, excrete sodium chloride when presented with an acute load, and maintain normal homeostasis after removal of the other kidney In these experiments the renal artery was sutured end to side to the common iliac artery, the renal vein was sutured end to end to the divided common iliac vein and the ureter was anastomosed to the bladder by a double flap technique (for details *see* Dempster, 1954)

Excellent function in kidneys transplanted autologously to the iliac region has also been reported by Murray, Lang and Miller (1955) and more recently by Bricker, Straffon Mahoney and Merrill (1958)

The iliac transplant is completely denervated and also isolated from the adrenal gland but differs from a transplant to the neck in respect of the method of dealing with the ureter This may account for the difference in function as Dempster *et al* suggested but it is conceivable that other factors for example environmental temperature may play some part

Dempster *et al* (1955) attributed the initial impairment of function of iliac transplants to the period of ischaemia to which the kidney was subjected during the operation, and thus supported the suggestion put forward eight years earlier by Hamburger (1947) Mitchell and Woodruff (1957) came to the same conclusion in the light of experiments in sheep in which one kidney was removed and then replaced in the loin with end to end anastomosis of the renal artery to the divided external iliac artery, end to side anastomosis of the renal vein to the inferior vena cava, and end to end anastomosis of the ureter over a polyethylene tube introduced via the bladder by means of a small cystoscope * They showed further that the effect of ischaemia was reduced if, during the time the blood supply was cut off, the kidney was kept at a temperature between 10° and 20°C † In these last experiments the kidney was exteriorized and invaginated into a plastic bag through which cold saline was passed The renal vessels were occluded temporarily with Blalock clamps care being taken not to compress or kink the ureter The temperature of the renal parenchyma was recorded by means of a needle thermocouple After two hours the Blalock clamps were released, the kidney was replaced in its normal site, and the opposite kidney was removed It was found that when the renal temperature was greater than 25°C all the animals subsequently passed small quantities of urine containing protein, blood and casts; showed a marked increase in blood urea and other manifestations of uraemia, and died within two weeks With temperatures from 20° to 25°C some animals became uraemic and died, but with temperatures from 10° to 20°C all the animals passed abundant urine of normal specific gravity and urea content which contained no protein, their serum electrolytes and blood urea remained normal, and they survived in good health until deliberately sacrificed The indigo carmine excretion test was also normal

*Only female sheep were used in these experiments

The Normal Behaviour of Homotransplants

The observations of Wu and Mann, and their colleagues at the Mayo Foundation (*v supra*), have been confirmed and extended by many investigators, including Hamburger (1947) and Oudot (1948a) in France, Lefebvre (1946, 1949a, b, 1950, 1952, 1953) in Belgium, Dempster (1950 1951, 1953a, d, 1954, 1955) in England, and Simonsen and his colleagues (Simonsen and Sørensen, 1949, Simonsen, Buemann, Gammeltoft,

†This does not conflict with the earlier work of Hamburger (1947) who showed that cooling to 0°C was of no benefit but did not study the effect of milder degrees of cooling

Jensen and Jørgensen, 1953; Simonsen, 1953a) in Denmark, and Hume, Miller and others in America (Parkinson and Woodworth, 1947; Murray, Favour, Wemyss and Miller, 1953; DeKlerk, Scott and Scott, 1954; Hume and Egdahl, 1955; Murray, Lang and Miller, 1955).

The findings are to some extent complementary, but where they do overlap they do not conflict in any important respect if due allowance is made for differences in operative technique and in the species of animal used. They may be summarized under the following headings: the function of homotransplants, arteriographic changes, gross structural changes, histological findings, and metabolic changes (other than those due to inadequate renal function) occurring in the host.

Function of Homotransplants. In dogs it was found that, irrespective of whether the transplant was placed in the neck and cutaneous ureterostomy was established (Oudot, 1948a; Lefebvre, 1949a, b; Dempster, 1950, 1951, 1953a, 1954, 1955), or the transplant was placed in the loin or groin and its ureter was anastomosed to the ureter or bladder of the host (Oudot, 1948a; Simonsen *et al.*, 1953; Hume and Egdahl, 1955; Murray *et al.*, 1955), urine usually began to flow within an hour, and often within 15 minutes, of re-establishment of the circulation, and continued to do so for a period which was usually between 2 and 12 days, being most commonly about 5 days, but was occasionally (Lefebvre, 1949a, b) as long as 19 days. At no time, however, did a homotransplanted kidney function as well as a successful autotransplant made by the same technique. In particular, homotransplants did not concentrate NaCl under conditions of salt loading as well as autotransplants, and animals which received a homotransplant immediately after both their own kidneys had been removed showed a steady rise in blood urea, though they appeared well and passed copious amounts of urine of specific gravity 1015-1020 for a few days. According to Lefebvre (1950, 1953), however, if homotransplantation was performed a day or two after bilateral nephrectomy, the blood urea, which was already elevated, began to fall, and if the alkali reserve was diminished it began to increase.

A few homotransplants in Dempster's series were anuric *ab initio*, but the same phenomenon occurs occasionally with autotransplants, and according to Dempster (1954) is probably due either to vasospasm caused by trauma to the nerves of the transplant at the time of operation or to damage to the filtering mechanism of the kidney.

If, as in some of Dempster's experiments, the recipient retained one or both of its own kidneys, the transplant secreted less urine than when both host kidneys were removed. Uraemia of course did not occur, but some animals developed a peculiar toxic syndrome characterized by anorexia, lassitude, pyrexia and hypertension. This syndrome was prevented by administration of terramycin and also by the development of uraemia—it thus never occurred when both host kidneys had been removed—and the symptoms disappeared within 12 hours if the transplant was removed. Dempster attributed it to blood-borne infection in a transplant already damaged by the host reaction; the evidence for this is not absolutely convincing, however, and it is of interest that a somewhat similar syndrome occurs in animals joined by parabiosis (p. 48).

Simonsen (1953a) reported that two kidneys which were returned to the donor 3 or 4 days after homotransplantation soon ceased to function. Dempster (1955), in a more extensive study of this phenomenon, found that kidneys returned to the donor after having been in another dog for 24 hours survived and functioned indefinitely, whereas those returned after 48 hours stopped secreting a few days later.

In goats Parkinson and Woodworth (1917) found that renal homotransplants in the neck secreted for about 10 days whereas Humphries (1957) observed secretion for 8–27 days (mean 15·6 days). Humphries suggested that the relatively prolonged functional activity in his experiments might have been associated with the fact that his goats were all castrated males but reported that he had not yet investigated this experimentally.

Arteriographic Changes Dempster (1953) found in dogs that immediately after transplantation the branches of the renal artery appeared sinuous in an arteriogram whereas 4 days later—while the kidney was still functioning—they were stretched as a result of enlargement of the kidney and no longer appeared sinuous. After the onset of *anuria* he observed *cortical ischaemia* associated with generalized spasm of the vessels.

The significance of this spasm is uncertain. Dempster (1953a) had suggested previously that afferent arteriolar spasm was the main factor in causing arrest of function in renal homotransplants but abandoned this hypothesis when he found (Dempster 1954) that in cortisone treated hosts anuria occurred in the presence of a good blood flow through the transplant.

Gross Appearance Homotransplants in dogs examined after 3 or 4 days were found to have increased in weight by about 60 per cent and like autotransplants showed swelling of the pelvis and ureter. They bled freely if incised. With transplants to the neck Dempster found no evidence of adhesion to surrounding host tissues but with transplants to the loin Simonsen found omentum adherent to the transplant almost invariably.

In the terminal stage homotransplants appeared bluish in colour, did not bleed freely when incised, and were found to have increased in weight two-fold or even three-fold. The capsule was thickened and stripped easily leaving a smooth surface. On section the cortex was wider than normal and had a somewhat nodular appearance. The medulla showed no gross changes other than slight oedema and some blurring of the parenchymal markings.

Histological Findings The histological findings have been studied in great detail in dogs by Dempster and Simonsen. Cellular infiltration appeared first in the cortex especially around the glomeruli and small blood vessels usually on the second or third day. Initially the cells were almost all mononuclears but later polymorphs appeared. Some of the mononuclear cells appeared to be small lymphocytes but many were found to be pyroninophilic when stained with pyronin methylene green (Unna Pappenheim stain) and were apparently plasma cells; initially these were of immature type but later mature plasma cells were seen. Mitoses were fairly numerous among the pyroninophilic cells and Simonsen claimed that a continuous transition could be discerned between resting non-pyroninophilic reticulum cells and active pyroninophilic cells. He therefore concluded that the plasma cells did not come from the host but originated in the transplant. In the later stages cellular infiltration was widespread throughout the cortex and was also seen in the fat of the sinus renalis and in the wall of the ureter; it was however rarely marked in the medulla.

The glomeruli appeared well preserved even in the terminal stages but sometimes showed some thickening of the basement membrane. The brush border of the proximal tubules began to fade about the third day and the distal tubules became dilated; later droplet degeneration occurred and numerous hyaline and granular casts were seen in the tubules.

The renal artery showed no evidence of damage: the endothelium was not swollen nor was it pyroninophilic and there was no

cellular infiltration in the vessel wall. On the other hand the intra-renal vessels, and the vessels of the capsule, sinus renalis fat and ureter, showed changes which Simonsen described as resembling those seen in periarteritis nodosa, and their endothelium was swollen and markedly pyroninophilic.

In the terminal stages the histological picture was one of widespread haemorrhage, oedema and necrosis, more pronounced in the cortex than the medulla, but, as we have seen, leaving the glomeruli relatively unscathed.

In addition to ordinary histological methods the technique of nephron dissection has been applied to renal homotransplants by Darmady, Dempster and Stranack (1955). This has revealed narrowing and loss of epithelium in the first part of the tubules in anuric transplants, and sometimes also in transplants removed before secretion ceased.

Examination of the organs of recipients of renal homotransplants other than the kidney has usually revealed no characteristic changes other than those attributable to hypertension or uraemia, but in three animals Simonsen observed an increased number of pyroninophilic cells in the spleen

Metabolic Changes in the Host. DeKlerk, Scott and Scott (1954) observed a sudden and sustained fall in the number of circulating eosinophils and also in the serum Na^{+}, and these changes occurred even if one of the host's kidneys was left *in situ*. The eosinopenia was attributed to increased adrenocortical activity, and the hyponatraemia to intracellular retention of sodium When the host's own kidneys were both removed there was an increase in the serum K^{+} in the terminal stages which was regarded as a manifestation of renal failure.

Nature of the Resistance to Renal Homotransplants

Modern views on transplantation immunity have developed mainly as the result of experiments with free homotransplants of skin and other tissues. It has been found, however, that the behaviour of homotransplants of whole kidneys, though differing in detail owing to the fact that they are provided with a blood supply *ab initio*, is governed by the same general laws.

The relevant experiments have been considered in detail in previous chapters. They include:

1. Experiments with successive renal transplants from the same or different donors (Dempster, 1953a, 1955; Simonsen, 1953a; Simonsen *et al.*, 1953), discussed in Chapter 5.

2. Observations on the behaviour of renal homotransplants in animals which had previously received homotransplants of skin (Dempster, 1953b; Simonsen *et al.*, 1953) or spleen (Simonsen *et al*, 1953), and *vice versa*, and observations on the behaviour of renal transplants in animals which had been connected by cross-circulation (Egdahl and Hume, 1956, 1957), discussed in Chapter 5.

3. Attempts to demonstrate circulating antibodies in recipients of renal homotransplants (Dempster, 1953a; Simonsen, 1953a), discussed in Chapter 5.

4. Observations on the effect of various procedures, including administration of cortisone (Persky and Jacob, 1951, Dempster, 1953c; DeKlerk, Scott and Scott, 1954), ACTH (Persky and Jacob, 1951) and nitrogen mustard (Baker, Gordon, Huffer and Miller, 1952), total body irradiation (Dempster, 1953a; DeKlerk *et al.*, 1954, Baker and Gordon, 1955), induction of uraemia in the host (Murray, Lang and Miller, 1955), and isolation of transplant from the regional lymphatics of the host by enclosing it in a plastic bag (Hume and Egdahl, 1955), discussed in Chapter 6.

5. Observations on the behaviour of renal homotransplants in hosts showing specific immunological tolerance to the tissues of the donor (*see* Simonsen, 1955), discussed in Chapter 7.

Clinical Observations

Homotransplants

The published cases of homotransplantation of the human kidney, apart from those in which the recipient was given total body irradiation or an identical twin was used as the donor (p 534), are summarized in Table IV.

The first case was reported by a Russian surgeon, Voronoy (1936). The recipient was a girl of 26 who was suffering from mercury poisoning. The transplant was obtained from a man who died of encephalitis, and was anastomosed to the femoral vessels of the recipient. The donor belonged to blood group B and the recipient to group O. The period for which the graft was ischaemic is not known. The transplant secreted clear urine and peristalsis was observed in the ureter. On the day after operation the patient was given a transfusion of group A blood.* Following this the urine became blood stained and the patient had convulsions. Next day urine secretion ceased and 48 hours after transplantation the patient died. Voronoy considered that the failure of this transplant might have been caused by the blood transfusion. It seems likely that this explanation is correct and that the transfusion also precipitated the patient's death.

Nine years later a second attempt to treat acute renal failure by means of a homotransplant was made by Landsteiner and Hufnagel (quoted by Hume, Merrill, Miller and Thorn, 1955). In this case the vessels of the transplant were anastomosed to the patient's brachial artery and cephalic vein. The patient's own kidneys resumed function after 48 hours and the transplant, which had not functioned, was removed. The transplant was obtained from a cadaver but again the period through which it was ischaemic is not known.

In 1950 Lawler, West, McNulty, Clancy and Murphy published a preliminary report of the first attempt to transplant a kidney to a patient whose renal function was impaired but who was not suffering from acute renal failure. The recipient was a woman aged 44 with bilateral polycystic kidneys. Her left kidney was removed and replaced by a transplant from a woman of 49 with the same blood type (AB Rh−) who had just died of haemorrhage from oesophageal varices. The vessels of the transplant were anastomosed end to end to the renal artery and the renal vein of the host with silk sutures. The ureter of the transplant was then anastomosed end to end to the host's ureter over a small catheter, the upper end of which was brought through the wall of the renal pelvis and then out through the incision. Once again the period for which the transplant was ischaemic is not known. The patient made a good recovery and 52 days after the operation an indigo carmine test showed that the transplant was functioning. It was found, however, that there was partial stricture of the ureter and 63 days after transplantation a second operation was performed with the intention of dealing with this stricture. The transplant appeared healthy and had a good blood supply, but there was a small abscess in the perirenal fat and on this account treatment of the ureteric stricture was deferred. The patient at this time was in excellent general health and her blood urea was 35 mg per 100 c.c.; little significance could be attached to this, however, in view of the fact that the patient's own right kidney was still functioning to some extent. When the graft had been in place for 9½ months it was again explored (Lawler *et al.*, 1951), but this time the findings were very different. The transplant still appeared to have a *blood supply* but was not functioning; it had become

*It seems very odd that a group O recipient should be given group A blood. In the Spanish version of Voronoy's paper, which is the only one the author has been able to trace, the recipient is described as a "donante universal (grupo O)", but some error may have occurred when the paper was translated from Russian to Spanish.

AN

given total body irradiation)

Period of Ischaemia of Transplant (minutes)	Haemodialysis	Result: Complications	Function of Transplant	Length of Survival of Patient after Transplantation	Structural Changes in Transplant
	No		Did not function	48 hours	
	No		Did not function Removed after 48 hours	Patient's own kidneys resumed function after 48 hours	
	No		Difficult to assess but transplant gave positive indigo carmine test 52 days after transplantation Not functioning when explored after 9 months	Still alive (patient's own R. kidney present)	
200	No		Functioning up to time of patient's death	20 days Died following intravenous injection of saline	Small infarcts Glomeruli congested Tubular degeneration Round cell infiltration Anastomoses patent
110	No	Necrosis of ureter	Kidney functioning up to time of patient's death, but ureterostomy unsatisfactory	17 days	Necrosis of ureter Ascending infection of kidney but no other abnormality observed
	No		Functioning 6 days after transplantation No subsequent report		

TABLE IV (Continued)

Source and Year of Report	Donor			Recipient		Technique					Result				
	Age Sex & Blood Type	*How Kidney Obtained*	*State of Donor Kidney*	*Age Sex & Blood Type*	*Disease*	*Host Vessels Used and Type of Anastomosis* — *Artery*	*Host Vessels Used and Type of Anastomosis* — *Vein*	*Method of Dealing with Ureter*	*Period of Ischaemia of Transplant (minutes)*	*Haemodialysis*	*Complications*	*Function of Transplant*	*Length of Survival of Patient after Transplantation*	*Structural Changes in Transplant*
Hume *et al.*, 1952 1953 1955	49 M A Rh+	Nephrectomy for tarcinoma of ureter	Normal	37 M A Rh+	Chronic pyelonephritis with hypertension and uraemia	Splenic after splenectomy end to end	L renal after L nephrectomy end to end	*Pelvis of* transplant anastomosed to host ureter	70	Pre op Post op on 9th 18th *20th and* 32nd days	Wound infection	Did not excrete dye or maintain patient	37 days	Perinephritic abscess, otherwise macroscopically normal Micro *glomeruli* relatively blood less but other wise normal Degeneration *of tubular* epithelium
	44 M O Rh+	Cadaver Donor died *during operation* for hypertension	B U N elevated Albuminuria	30 F A Rh+	Pyelonephritis hypertension chronic uraemia	*Profunda* femoris end to end	Superficial femoral end *to end*	Cutaneous ureterostomy	150	Pre op *Post op* on 2nd day	Huge haematoma due to erosion of femoral artery	Did not *function* Removed after 37 days	25 days	Total infarction
	47 F B Rh+	Cadaver Donor died during operation for mitral *stenosis*	Albuminuria B U N normal *Micro* exam other *kidneys showed* multiple small infarcts	43 F B Rh+	Chronic pyelonephritis hypertension uraemia	Profunda *femoris* end to end	Common femoral end to end	*Cutaneous* ureterostomy	135	Pre op Post op on 57th day	Skin sloughed over trans *plant*	Anuric for 8½ days *then* good output to 33rd day, *fair 33rd* to 53rd day, and *still some* excretion to 73rd day	99 days	Vascular thrombosis Autolysis of transplant
	21 M O Rh+	*Cadaver* Donor died *following* operation for tetralogy of Fallot	Slight albuminuria B U N normal	52 F O Rh+	Polycystic kidneys *pyelo* nephritis hypertension uraemia	Profunda femoris end to end	*Common* femoral end to side	Cutaneous ureterostomy	200	Pre op Post op on 98th and 100th days		Anuric for 11 days Maximum function *13th 28th day*, declining slowly 29th 65th day and rapidly 65th *101st day* Transplant removed 105th day	7 months	Vascular thromboses *Multiple* abscesses and areas of *infarction*
	4½ F O Rh+	Nephrectomy to permit subarachnoid ureteral anastomosis for hydrocephalus	Normal	18 F O *Rh+*	Chronic glomerulonephritis hypertension uraemia	*Profunda* femoris *end to end*	Common femoral *end to side*	Cutaneous ureterostomy	55	No	Infected haematoma	*1700 ml* urine excreted first 24 hours Almost anuric 2nd 13th days *then good* function 14th 26th days after which declined Function ceased after 37 days	47 days	Haemorrhage abscesses, and areas of infarction

TABLE IV (Continued)

Source and Year of Report	Donor			Recipient		Technique					Result			
	Age, Sex & Blood Type	How Kidney Obtained	State of Donor Kidney	Age, Sex & Blood Type	Disease	Host Vessels Used and Type of Anastomosis: Artery	Host Vessels Used and Type of Anastomosis: Vein	Method of Dealing with Ureter	Period of Ischaemia of Transplant (minutes)	Haemodialysis	Complications	Function of Transplant	Length of Survival of Patient after Transplantation	Structural Changes in Transplant
Hume et al (Cont'd)	[illegible] F O Rh+	Nephrectomy to permit ventricular ureteral anastomosis for hydrocephalus due to tumour	Normal	24 M O Rh+	Chronic pyelonephritis uraemia	Profunda femoris end to end	Common femoral end to end	Cutaneous ureterostomy	11	Post op 4th 14th and 49th days	Haematoma	700 ml urine excreted first 12 hours Thereafter anuria Removed after 52 days	57 days	Biopsy 19 days showed marked ischaemia, interstitial round cell infiltration, degeneration of tubular epithelium At 57 days extensive vascular thrombosis, infection and necrosis
	[illegible] F B Rh+	Cadaver Died during operation for auricular septal defect	Albuminuria B U N normal Aberrant artery	28 F O Rh+	Chronic pyelonephritis, hypertension, congestive cardiac failure, uraemia	Profunda femoris end to end	Common femoral end to side	Cutaneous ureterostomy	200	Pre op Post op 5th day (also peritoneal lavage post op 4th, 15th and 17th days)	Haematoma Severe hyperkalaemia	Did not function Removed 17th day	19 days	Total infarction
	[illegible] M O Rh+	Cadaver Donor died during operation for ventricular septal defect	Albuminuria and some casts B U N normal Donor had cardiac failure	57 F O Rh+	Periarteritis nodosa, with destruction of kidneys and anuria	Profunda femoris end to end	Common femoral end to side	Cutaneous ureterostomy	275	Pre op Post-op on 6th and 16th days		Did not function	38 days	Microscopic changes resembling severe acute glomerulo nephritis

TABLE IV (Continued)

Source and Year of Report	Donor			Recipient		Technique					Result			
	Age Sex & Blood Type	How Kidney Obtained	State of Donor Kidney	Age Sex & Blood Type	Disease	Host Vessels Used and Type of Anastomosis: Artery	Host Vessels Used and Type of Anastomosis: Vein	Method of Dealing with Ureter	Period of Ischaemia of Transplant (minutes)	Haemodialysis	Complications	Function of Transplant	Length of Survival of Patient after Transplantation	Structural Changes in Transplant
Hume *et al* (Cont d)	97 F A Rh+	Cadaver Donor died during cardiac operation	No albumin B U N normal Donor had cardiac failure	26 M A Rh+	Chronic glomerulonephritis severe hypertension uraemia	Profunda femoris end to end. Kidney surrounded by a polythene bag	Common femoral *end to side*	Cutaneous ureterostomy	180	No	Haematoma	Anuric for 11 days and daily excretion only 5 20 ml from 12th to 18th day There after improved reaching maximum output of 1 3 litres from 37th 170th day	176 days P M pericarditis pleural effusion pulmonary oedema and atelectasis	Anastomoses patent Polythene bag had torn exposing both poles of kidney Micro some interstitial *fibrosis* and round cell infiltration *glomeruli* somewhat ischaemic, *marked* intimal sclerosis of intrinsic blood vessels some atrophy of tubular epithelium
Murray & Holden 1954		Cadaver		F	Chronic glomerulonephritis	External iliac *end to side*	External *iliac* end to side	*Anastomosed* to bladder	150	No		Difficult to assess	Alive and working at time of last report (15 months after transplantation)	
Joekes *et al*, 1957	52 M A Rh+	Cadaver Donor died during operation		22 F A Rh+	Septic abortion Acute renal failure	Superficial femoral end to end	Superficial *femoral* end to end	Cutaneous ureterostomy	170	No		Secretion limited to 100 ml or less of blood stained urine daily	5 days	Proximal tubular necrosis with evidence of regeneration

very much smaller, and the ureter and pelvis were completely obliterated.

In 1951 no less than eight cases in which renal homotransplants were used were reported by French workers

Servelle, Soulié, Rougeulle, Delahaye and Touche (1951a, b) transplanted a kidney from an executed criminal to a girl aged 20, who was suffering from congenital absence of the right kidney, and nephritis in the left kidney associated with hypertension. The blood groups of the donor and host are not recorded The transplant was perfused with Ringer's solution from the time it was removed from the body of the donor until the start of the operation. It was ischaemic for a total period of 3 hours 20 minutes The artery of the transplant was anastomosed end-to-side to the patient's external iliac artery, the vein was anastomosed to the end of the divided iliac vein, and a cutaneous ureterostomy was established The transplant began to secrete urine almost as soon as the circulation was restored, and the amount secreted increased to a maximum of 600 c c. per 24 hours on the 19th day after operation The concentration of urea in the urine from the transplant ranged from 4 to 7 g per litre and the concentration of chloride remained fairly constant at approximately 4 g per litre. Sixteen days after transplantation the patient was given 250 c c of isotonic saline solution subcutaneously and immediately afterwards went into a state of severe shock which lasted for several hours Despite this a further injection of saline was given on the 19th day and was followed by severe shock and death. At postmortem examination the anastomosis was patent and macroscopically the graft appeared healthy except for a small area of infarction. Histological examination revealed multiple very small infarcts, which appeared to be in process of being absorbed. The glomeruli were congested, the tubules showed changes typical of acute nephritis, and there was abundant interstitial lymphocytic infiltration.

DuBost and his colleagues (DuBost, Oeconomos, Vaysse, Hamburger, Milliez and Lebrigand, 1951; DuBost, Oeconomos, Vaysse, Hamburger, Nenna and Milliez, 1951) transplanted a kidney from an executed criminal to a woman aged 44, one of whose kidneys had been removed for tuberculosis and whose other kidney was grossly disorganized as a result of chronic pyelonephritis. The recipient belonged to blood group A and was Rh−, the donor also belonged to blood group A but was Rh+. The procedure of removing the kidney from the body was started 15 minutes after death and was completed in a further 15 minutes. The transplant was perfused with plasma for 5 minutes and was then transported to the hospital in a constant temperature box containing Tyrode's solution and antibiotics. The vessels of the transplant were anastomosed end-to-side to the patient's common iliac artery and vein, and a cutaneous ureterostomy was established. The period for which the transplant was ischaemic was 1 hour 50 minutes. The patient received 25 mg. of ACTH during the operation and 50 mg. daily thereafter. In addition she received 25 mg. of cortisone four times daily after operation, and also penicillin and streptomycin. At the end of the operation the transplant began to secrete blood-stained urine. The urine remained blood-stained until the third day, after which it appeared clear but still contained albumin For the first six days the amount of urine secreted by the transplant was only 50 c.c. daily, but thereafter it increased gradually to a maximum of 200 c c. daily, and the concentration of urea, which had initially been only 4 g per 100 c c. increased to 14 g. per 100 c.c. In spite of this the patient's blood urea increased, and on the 12th day exsanguination-transfusion was performed. On the 16th day only a small quantity of urine was passed, and this was

turbid owing to the presence of pus and phosphates The ureterostomy did not appear to be functioning satisfactorily and on exploration it was found that the distal 5 cm of the ureter was gangrenous The gangrenous portion was removed and the ureterostomy was refashioned Following this procedure the patient passed copious blood stained urine, but her condition deteriorated and she died on the following day At post mortem examination the anastomoses were patent Part of the ureter was again found to be gangrenous but the graft otherwise appeared macroscopically normal Histological examination of the graft revealed evidence of ascending infection but no other abnormality was observed

DuBost and his colleagues refer briefly to a second patient in whom they performed homotransplantation of a kidney The transplant appears to have been functioning satisfactorily six days after operation, but its subsequent behaviour is not recorded

Kuss, Teinturier and Milliez (1951) performed renal transplantation in five patients

The first recipient was a woman aged 14 who had had one kidney removed some years previously for a stag horn calculus and was in a state of chronic uraemia owing to the presence of calculi and gross infection in the remaining kidney The transplant was obtained from a woman aged 34 of the same blood type (O, Rh+) as the recipient who was complaining of recurrent renal colic and on investigation was found to have a stricture of the right ureter At operation a plastic procedure did not appear to be feasible and the kidney was therefore removed The vessels of the transplant were anastomosed end to end to the divided internal iliac artery and external iliac vein of the patient, and a cutaneous ureterostomy was established The transplant was ischaemic for 1 hour 15 minutes After operation the patient received ACTH (50 mg daily), Phenergan and antibiotics The graft began to secrete urine immediately the clamps were released, but the amount passed remained small and never exceeded 45 c c per 24 hours Twenty days after operation it was observed that the ureterostomy was not functioning properly and necrosis was observed at the orifice Shortly after this the patient had an episode of haematuria and her condition began to deteriorate rapidly She died 35 days after operation and at post mortem examination the ureter was found to be completely necrotic There was an area of infarction in the transplant but the anastomoses appeared to be patent

The second case is of special interest in that the recipient was suffering from bilateral renal tuberculosis and the transplant was itself a tuberculous kidney obtained from a patient whose other kidney appeared to be free of the disease The recipient was a man aged 22 and the donor was a girl of the same age and blood type (A, Rh+) The technique of anastomosis was the same as *in the previous case, and the period for* which the graft was ischaemic was 1 hour 25 minutes Following the operation the recipient was given antibiotics and Phenergan but no ACTH The transplant secreted 15 c c of urine during the first 24 hours after operation and thereafter the volume gradually increased until it reached a maximum of 72 c c per 24 hours Two months after operation the patient was sufficiently well to leave hospital His condition was still reasonaby good 3½ months after operation, and his blood urea was only 70 mg per 100 c c, but the transplant was now excreting only a few c c of urine daily

The third patient was a woman of 48 with bilateral ureteric obstruction caused by widespread carcinoma of the cervix The transplant was obtained from a woman aged 40, of the same blood type (A, Rh+) as the recipient, who was suffering from hypertension and whose right kidney showed some degree of hydronephrosis This kidney was removed and, despite the fact that

it possessed an aberrant renal artery which had to be ligated, it was considered suitable for use as a transplant. The recipient's internal iliac artery was involved in tumour tissue, and the main artery of the transplant was therefore anastomosed end-to-side to the patient's external iliac artery. The vein of the transplant was anastomosed end-to-end to the divided external iliac vein of the patient, and cutaneous ureterostomy was established as in the previous cases. The transplant was ischaemic for only 45 minutes. It began to secrete urine as soon as the clamps were released. Fifteen days after operation the output was 80 c.c. daily, and a pyelogram performed at this time revealed no abnormality, but three days later the transplant stopped secreting. The patient's condition declined rapidly and she died 20 days after operation. Post mortem examination revealed an infarct of the upper pole of the transplant, but the rest of the parenchyma appeared practically normal and there was no necrosis of the ureter.

The fourth patient, a boy of 15 suffering from bilateral congenital hydronephrosis, died during the operation of transplantation.

The last patient, a young man of 22 who was also suffering from bilateral hydronephrosis, received a kidney from an executed criminal. The transplant was not very satisfactory owing to the fact that there were two arteries in the main renal pedicle and a third artery, separate from the main pedicle, going to the superior pole of the kidney, but as the recipient had been prepared it was decided to go ahead with the operation. The larger of the two arteries in the pedicle was anastomosed end to side to the patient's external iliac artery, and the main vein of the transplant was anastomosed end to end to the divided external iliac vein. Cutaneous ureterostomy was performed as in the other cases. The other vessels of the transplant were ligated. The transplant was ischaemic for only 1 hour 15 minutes, but despite this it failed to secrete and was removed after 18 hours, when it was found to be completely avascular.

In the cases considered so far either the transplant failed to secrete *ab initio*, or the patient died while the transplant was still functioning, or the cessation of function could reasonably be attributed to some obvious cause such as infection, thrombosis, necrosis of the ureter, or ureteric stricture. In the case now to be described, which was reported by Michon, Hamburger, Oeconomos, Delinotte, Richet, Vaysse and Antoine (1953),* the cessation of function could not be explained in this way, and there seems little doubt that it was a manifestation of homotransplant immunity. The case is remarkable also for the fact that the transplant was a normal kidney obtained from a voluntary donor, and for the very thorough study which was made of its functional activity and of the histological changes which occurred in it. The recipient was a boy aged 16 in whom nephrectomy was performed for traumatic rupture of the right kidney associated with severe haemorrhage. Following this operation he passed no urine whatever and on investigation it was found that he had no other kidney. His blood type was found to be B, Rh+ (C—D—ee), P+, MN, and his mother, whose blood type was B, Rh+ (CcD—ee), P+, N, offered to donate one of her kidneys for use as a transplant. This offer was accepted on the grounds that transplantation offered the only possible hope of saving the boy's life, and the operation was performed 7 days after the nephrectomy. During this interval the patient received a low-protein high caloric diet, and a low fluid intake. In consequence he had no vomiting, diarrhoea or other symptoms of uraemia, and was well able to stand the operation.

The vessels of the transplant were anastomosed to the patient's external iliac

*See also Oeconomos, Hamburger, Delinotte, Vaysse, Richet, Antoine, Hinglik and Petrin (1953)

artery and vein and the ureter of the transplant was anastomosed to the stump of the patient's ureter over a catheter. After operation the patient received penicillin, streptomycin, vitamin B_{12} and repeated blood transfusions. The transplant was ischaemic for 55 minutes. It began to secrete urine three hours after operation and the rate of excretion rapidly increased until it was approximately one drop per second. During the next 24 hours the patient passed more than 3 litres of urine, and thereafter he passed approximately 1.5 litres per 24 hours until the 22nd day when the transplant suddenly stopped functioning and the patient became completely anuric. The urine at first contained abundant red cells and leucocytes but soon cleared. It also contained a little albumen and this persisted all the time the transplant was functioning. The urine was cultured daily but except on one occasion was always found to be sterile. The concentration of urea in the urine which was initially 1.2 g. per 100 c.c. increased to a maximum of 1.8 g. per 100 c.c. and then dropped to 1.5 g. per 100 c.c., the daily output of urea becoming stabilized at between 20 and 30 g. Shortly after transplantation when the diuresis was maximal the urea clearance was 11.2 c.c. per minute and subsequently became stabilized at 12 c.c. per minute. During the time that the transplant was functioning the blood urea fell steadily from 180 mg. per 100 c.c. to 80 mg. per 100 c.c. The diastolic and to a lesser extent the systolic, blood pressure increased steadily. The optic discs became increasingly pale but the vessels of the fundi showed little change. The serum globulin increased from 2.1 g. per 100 c.c. a few days after transplantation to 3.2 g. per 100 c.c. when the transplant ceased functioning, and paper electrophoresis showed that this was due mainly to an increase in gamma globulin. The bone marrow immediately prior to the transplantation showed gross erythroblastic aplasia but improved rapidly thereafter. For the first 15 days however, there was no corresponding improvement in the peripheral blood picture, and the patient was therefore given a daily transfusion of 300 c.c. of blood.

Various complications developed including one episode of acute haematuria, venous thrombosis of the right leg, haemorrhagic pleural effusion and diarrhoea, but 17 days after transplantation the patient's condition was remarkably satisfactory and he began to sit up in bed. On the afternoon of the 22nd day however, without any warning symptoms he passed only a small quantity of urine and thereafter was completely anuric. The transplant was explored and was found to have approximately doubled in size. It was violet in colour with patches of ecchymosis. The vessels and ureter were patent and no mechanical cause for the anuria was discovered. The patient was treated with a low protein high calorie diet, cortisone, phenergan, antibiotics and exsanguination transfusion but developed typical symptoms of uraemia and died 10 days later.

A biopsy was performed when the transplant was explored on the 22nd day, and this showed accumulation of lymphocytes and plasma cells, ischaemia of the glomerular capillaries with distension of vessels elsewhere, and degeneration and desquamation of tubular epithelium. Ten days later on post mortem examination it was found that disorganization of the transplant had progressed considerably. Many of the intrarenal and extrarenal vessels were thrombosed and there were corresponding areas of infarction. The appearance of the glomeruli resembled that seen in eclampsia and there was widespread degeneration and desquamation of the epithelium of the straight and convoluted tubules.

This case is instructive in many ways and the following points, to which Michon *et al.* drew attention, are of particular interest.

In the first place, it was found that a considerable amount of fluid had to be adminis-

tered in the early stages in view of the diuresis which occurred, and which threatened to disturb the patient's fluid and electrolyte balance.

Secondly, the correction of the patient's erythroblastic aplasia following transplantation was taken as evidence that the transplant in some way facilitated recovery of the bone marrow. Admittedly the patient received 100 μg of vitamin B_{12} each day, but according to Michon *et al.* this alone has little effect on the anaemia which occurs in patients with chronic uraemia.

Thirdly, the capacity of the transplant to clear urea and electrolytes, though remarkably constant after the first 24 hours until the terminal stages, was decidedly inferior to that of the other kidney remaining in the donor. Michon *et al.* suggested that this might be due to the period of ischaemia to which the transplant was subjected during the operation, but as this period was only 55 minutes it seems likely that the explanation lies elsewhere.

Finally, the sudden arrest of function of the transplant without obvious mechanical cause and the histological findings, considered in the light of animal experiments, suggest that the eventual failure of this transplant was due to an immunological reaction on the part of the host. Michon *et al.* suggested that the rise in gamma globulin which occurred in the host serum may have been due to the appearance of circulating antibody, but no direct evidence of this was obtained.

Hume and his colleagues (Hume, Merrill and Miller, 1952; Hawn, Hume, Merrill and Miller, 1953; Hume, Merrill, Miller and Thorn, 1955) reported nine clinical cases of homotransplantation of the kidney.

Six of the transplants were obtained from cadavers, two from patients with hydrocephalis in whom one kidney was being removed in order to establish drainage of cerebrospinal fluid via the corresponding ureter, and one from a patient with a carcinoma of the lower part of the ureter. Six of the recipients were suffering from chronic pyelonephritis, two from chronic glomerulonephritis, and one from periarteritis nodosa associated with anuria.

In one patient, who was operated on in another hospital before coming under the care of Hume and his colleagues, the artery and vein of the transplant were anastomosed to the splenic artery and the renal vein respectively after removal of the spleen and left kidney, and the pelvis of the transplant was anastomosed to the host's ureter with the aid of a T-tube. In the other patients the transplant was placed in a pocket in the patient's thigh, the renal artery was anastomosed end-to end to the divided profunda femoris, the renal vein was anastomosed end-to-end to the superficial or common femoral vein or end-to-side to the common femoral, and the stump of the ureter was brought out through the skin (Figs. 159, 160). In most cases a double-ended pedicle flap of skin and subcutaneous tissue was raised from the lateral part of the thigh and swung medially to provide cover for the transplant, the resulting defect being covered by a split-skin graft from the opposite leg. Post-operatively eight of the patients received heparin, six received ACTH or cortisone or both, all received testosterone,* and all received one or more of the following antibiotics: penicillin, streptomycin, terramycin, aureomycin and chloromycetin. Haemodialysis with an artificial kidney was performed pre-operatively in six patients, and on one or more occasions after operation in seven patients.

Four of the transplants developed measurable function. All these were in recipients who were of the same blood type as the transplant donor. Three were anuric for 8-11 days before they began to function; the other transplant functioned for 24 hours.

* Testosterone was given in view of Selye's accounts of its effect on the kidney (Selye, 1939, 1940, Selye and Friedman, 1941)

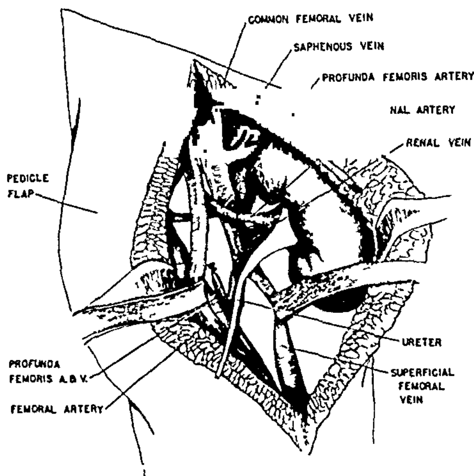


Fig 159 Method of placing a human kidney homotransplant in the thigh used by Hume Merrill Miller and Thorn The kidney is buried in a pocket created between the subcutaneous tissue and the muscles in the medial aspect of the thigh The renal artery is anastomosed end to end to the profunda femoris artery and the renal vein is anastomosed end to side to the common femoral vein A double ended pedicle flap is pulled over to help to cover the kidney without tension on the skin A split skin graft (not shown) is then applied over the lateral aspect of the thigh in the defect created by moving over the pedicle flap The ureter will be brought out through a stab wound in the lower portion of the pedicle flap (Reproduced from the *Journal of Clinical Investigation* by courtesy of the publishers and Dr David M Hume)

then became anuric for 11 days and then began to function again During the period of maximal performance each of the four successful transplants functioned better than the recipient's own kidneys

Apart from these similarities there were striking differences between the first three successful transplants and the fourth one

The first three received ACTH and cortisone post operatively Function was maximal between the second and fourth week after transplantation but declined rapidly thereafter The final destructive processes appeared to consist of cortical ischaemia tubular degeneration and interstitial oedema and round cell infiltration, followed by scattered thromboses of the small intrinsic renal vessels with associated focal infarcts (Fig 161) All three transplants ultimately became heavily infected The glomeruli appeared to be relatively normal except for ischaemia until the de



Fig 160 Method of collecting urine from a kidney transplanted to the thigh (Reproduced from the *Journal of Clinical Investigation* by courtesy of the publishers and Dr David M Hume)

struction of the kidney by infection was nearly complete The main renal vessels remained patent in one case, and had become thrombosed by the terminal stage in the other two cases

The fourth successful transplant was placed in a polyethylene bag at the time of transplantation by a technique similar to that used by Hume and Egdahl (1955) in dogs (p. 114). This was done in an attempt to isolate it from the local lymphatic drainage, in the hope of preventing or reducing antibody formation No ACTH or cortisone was given. Maximal function was not reached until the 37th day, and the function persisted without diminishing for over five months from the time of transplantation. The blood urea nitrogen diminished from 244 mg. per 100 ml. on the 19th day after transplantation to 31 mg. per 100 ml. on the 153rd day, and following the breakdown of the transplant rose again rapidly over a period of two weeks to reach 260 mg. per 100 ml. on the day the patient died. At autopsy the polyethylene bag was found to be intact over the central part of the kidney but was torn over the poles. Microscopically (Fig. 162) the tubules showed signs of acute ischaemic nephrosis, and there was interstitial oedema and focal round-cell infiltration as in the other cases. There was however no significant infection, and no thrombosis or infarction. The blood vessels showed marked intimal thickening. There was some glomerular damage in the outer layers of the cortex in that portion of the kidney which was covered by the bag, but none in the deeper layers of the cortex nor in parts of the kidney not covered by the bag.

From the whole series of cases Hume *et al.* drew the following conclusions:

1. It is possible for transplants which have been anoxic for 3-3½ hours to develop about 25 per cent of normal function.

2 Function extending over weeks or months may be preceded by a period of anuria lasting eight days or longer. This anuria is probably a consequence of the period of ischaemia to which the transplant is inevitably subjected.

3. Renal homotransplant rejection in man is much less violent and slower to develop than it is in the dog, though the general pathological picture which accompanies breakdown of the transplant in man is qualitatively similar to that seen in experimental animals.

4 It seems desirable that the donor and host should be of the same blood type, but the necessity for this has not been conclusively established in the present series of cases because in the two instances in which the donor and host were of different blood types the transplants were perfused before being connected to the host and this may have had a deleterious effect.

5. ACTH and cortisone do not appear to exert any profound beneficial effect on the survival of human renal transplants.

Hume *et al.* carefully refrained from drawing any conclusions about the effect of enclosing the renal transplant in an impermeable bag because this procedure was

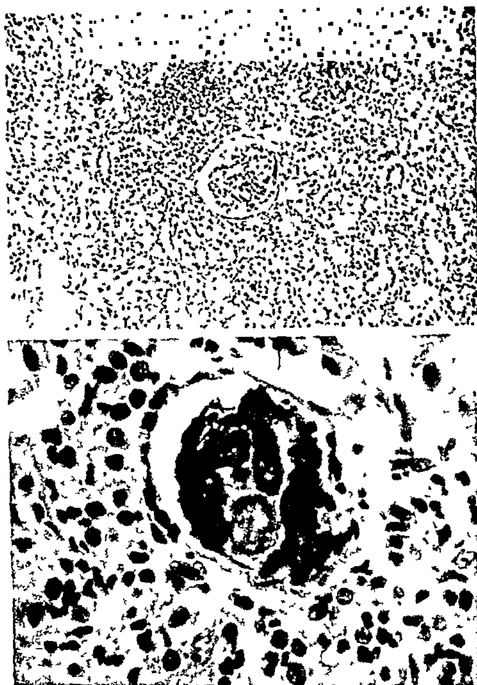


Fig 161 Biopsy of human kidney homotransplant after 70 days Above low power view (×150) showing evidence of marked infection with round cell and polymorphonuclear leucocytic infiltration of the interstitial spaces There is no evidence of acute or chronic glomerulonephritis The glomerulus in the centre of the picture is somewhat ischaemic but otherwise appears surprisingly normal Below higher magnification (×400) showing laminated basophilic casts in the tubular lumen Haematoxylin and eosin (Reproduced from the *Journal of Clinical Investigation* by courtesy of the publishers and Dr David M Hume)



Fig. 162. Section of kidney homotransplant which had been enclosed in a polyethylene bag after 180 days. The glomerular tufts appear ischaemic but otherwise fairly normal. There are no proliferative changes present in the glomerular capsule. The epithelium of the tubules is low and the nuclei are variable staining. There is interstitial oedema and polymorphonuclear and round cell infiltration. Haematoxylin and eosin × 300. (Reproduced from the *Journal of Clinical Investigation* by courtesy of the publishers and Dr David M. Hume.)

used in only one case and in this particular instance the bag did not remain impermeable. Experiments subsequently reported by Hume and Egdahl (1955), which have already been considered (Chapter 6), suggest that in dogs this procedure does not prolong survival of renal homotransplants, but in view of the different behaviour of such transplants in man it would seem worthwhile to undertake further clinical trials.

Murray and Holden (1954) performed renal homotransplantation in four patients, but three of them died. The fourth patient, who was suffering from chronic glomerulonephritis, was alive and apparently well 15 months after transplantation, and it was assumed that the transplant must be functioning, but no function studies or biopsies have been reported.

Joekes, Porter and Dempster (1957) reported what appears to be the first case of homotransplantation of a human kidney to be undertaken in Great Britain. The transplant was obtained from a man aged 52 who died during an operation, and the recipient was a young woman of 22 suffering from acute renal failure following a septic abortion. Both donor and recipient belonged to blood group A and were Rh+. The transplant was placed in the recipient's thigh,* the renal vessels being anastomosed to the superficial femoral artery and vein, and the ureter being brought out through the skin. The transplant was ischaemic for 117 minutes. It secreted a little blood-stained urine, but the quantity never exceeded 100 ml in 24 hours. The patient died five days after transplantation, and histological examination of the transplant showed proximal tubular necrosis with evidence of regeneration.

As we have seen in Chapter 22, Merrill (1958) and his colleagues treated one patient by total body irradiation and homotransplantation of bone marrow, followed by homotransplantation of a kidney. In the author's view, however, the lack of success was almost inevitable, owing to the fact that some of the marrow came from an adult donor other than the donor of the kidney.

Subsequently the same group of investigators (*see* Merrill, 1959, p. 11; Murray, Wilson, Dealy, Sadowsky and Corson, 1959, p. 368) transplanted a kidney from a non-identical twin donor after irradiating the recipient (400 r total body irradiation) but without transplanting bone marrow. The recipient's own kidneys were removed, and 8 months after the operation the transplant

* The operation was performed by C. G. Rob and W. J. Dempster.

was still functioning satisfactorily, although a biopsy revealed histological evidence of a *homograft reaction* The significance of this case is however somewhat obscured by the fact that the twins, though different in appearance, had no less than 23 red cell antigens in common

Still more recently Hamburger, Vaysse, Crosnier, Tubiana, Lalanne, Antoine, Auvert, Soulier, Dormont, Salmon, Maisonnet and Amiel (1959) have reported another case in which the function of a kidney transplanted to an irradiated recipient (460 r total body irradiation) from a non identical twin was about 80 per cent of normal four months after the operation

Isotransplants

It seemed likely from observations with skin grafts that a renal homotransplant might survive permanently if the donor and host were identical twins The truth of this surmise was demonstrated for the first time by Merrill, Murray, Harrison and Guild (1956) in the following remarkable case

The recipient was a young man of 24 who presented with oedema and hypertension, and was found on investigation to have extreme atrophy of both kidneys He happened to have a twin brother, and the hospital record of their birth showed that there had been a common placenta Both twins had Darwin's tubercles on their ears (whereas their siblings had not), showed identical eye colours, were able to taste phenylthiocarbamide, and were indistinguishable on the basis of eight different blood group systems It appeared almost certain therefore that the twins were identical, but to place the matter beyond doubt skin grafts were interchanged between them and these were found on biopsy examination 31 days later to present the appearance of normal skin The prospective twin donor was in perfect health, and was found on investigation to possess two kidneys, both of which showed normal function and no evidence of infection His offer to donate one kidney was therefore accepted, and the transplantation was performed 38 days after the skin grafts had been interchanged

The transplant was placed in the pelvis of the recipient on the right side (Fig 163) The main renal artery was anastomosed end to end to the divided internal iliac artery, and the renal vein was anastomosed end to side to the common iliac vein A small accessory artery in the renal pedicle was ligated The bladder was opened and the ureter of the transplant was drawn into the bladder through an intramuscular and submucosal tunnel, mucosa to mucosa anastomosis between ureter and bladder was then performed A polyethylene catheter was passed through the anastomosis into the pelvis of the transplant, and the lower end of the catheter was brought out through the cystostomy wound to the surface The incision in the bladder was closed after a mushroom catheter had been inserted through a suprapubic stab Clear urine was then seen to be flowing copiously from the ureteral catheter The kidney appeared to be lying rather neatly in its new site except that it projected forward where the lower pole impinged upon the iliac crest It was anchored in place by sutures, and the incision was then closed in layers The kidney was ischaemic for 88 minutes, and the total operating time was 3½ hours The post operative course was smooth, and the incision healed *per primam* The ureteral catheter was removed on the ninth post operative day after evidence of function had been confirmed by the prompt excretion of injected indigo carmine

The patient continued to improve and left hospital 37 days after the operation In this time he had lost his oedema, but had nevertheless put on 11 lb in weight and his appetite was good An electrocardiogram revealed no abnormality, and his heart had resumed its normal size His blood pressure, which before operation had

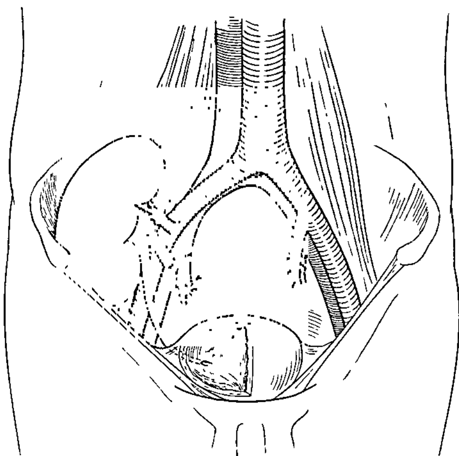


Fig 163 Connexions of the kidney transplant from an identical twin donor in the patient reported by Merrill, Murray, Harrison and Guild (Reproduced from the *Journal of the American Medical Association* by courtesy of the publishers and Dr John Merrill)

been 220/146 mm Hg, was 120/60 mm. Hg, and his blood urea nitrogen, which had been 185 mg per 100 ml, was now only 14 mg. per 100 ml. His serum electrolytes were normal, and the serum CO_2 combining power was 25 mEq./l.

During the follow-up visits however it was found that the blood pressure was often a little elevated, and the urine contained leucocytes and grew a variety of organisms on culture. Since intravenous pyelography (Fig. 164) and other function tests confirmed that the transplant was functioning satisfactorily whereas the patient's own kidneys were not, it was decided to perform bilateral nephrectomy, and this was done at two separate operations three months and five months respectively after transplantation. Following these procedures the blood pressure fell to and remained within normal limits, the urine ceased to show evidence of infection, and the patient resumed full activity. He was still in excellent health 12 months after the transplantation had been performed.

Subsequently, Murray, Merrill and Harrison (1958) have given further information about the progress of this patient, and have reported six more cases in which a kidney was transplanted from an identical twin by a similar technique, the only difference being that in four patients the anastomosis between the ureter and the bladder was not splinted.



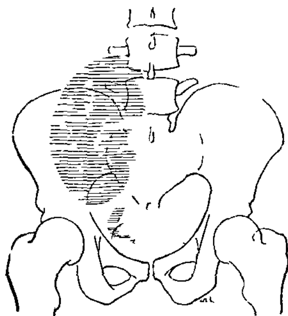


Fig 164 Intravenous pyelogram in patient reported by Merrill Murray Harrison and Guild The transplant appears to be functioning satisfactorily but the patient's own kidneys showed no evidence of function (Reproduced from the *Journal of the American Medical Association* by courtesy of the publishers and Dr John Merrill)

All seven donors were reported to be alive and well though one had had some infection of the wound The transplants functioned in six of the recipients and in five of the six began to secrete urine almost immediately One recipient died four months after transplantation, one developed nephritis in the transplant and four were reported to be alive and well

A further successful case in which a kidney was transplanted from an identical twin has been reported briefly by Hume (1958)

THE PROBLEM OF STORAGE

Lefebvre (1951) perfused dogs kidneys via the renal artery with physiological saline at a controlled temperature (1°–22°C) and pressure (14–16 mm mercury) until the liquid coming from the vein appeared free of blood and then stored them at temperatures ranging from 1° to 10°C for 3–24 hours, prior to connecting them to the carotid jugular circulation of other dogs as homotransplants

In many cases the renal circulation was successfully re established and the transplant began to secrete urine after 15–30 minutes The amount secreted was usually small in normal hosts but somewhat greater in hosts which had previously been nephrectomized One transplant in a nephrectomized host for example which had been stored for 24 hours at 5°C secreted 81 c c of urine in 2¼ hours The transplants were able to excrete chloride and bile pigment, but concentration of urea was feeble

Three kidneys which failed to secrete any urine when transplanted after storage by the method described above were examined histologically by Lefebvre and Nizet (1952), two of them after injection of encre de Chine The findings pointed to the existence of an intrarenal vascular shunt ... pletely excluding ... appa...

mediated by connexions between the arcuate arteries and veins.

In these experiments the kidneys were observed for at most a few hours after transplantation Long-term studies are required, however, to assess the merits of a method of storage because it is essential to determine whether a transplant is capable of resuming satisfactory function if allowed time for recovery, and in such investigations it is clearly advantageous to use autotransplants, at any rate in the first instance

The author plans to study the effect of continued perfusion at reduced temperatures with oxygenated heparinized blood or a suspension of erythrocytes, using both constant and pulsatile flow, and also the effect of enclosing a kidney, together with a simple form of bubble oxygenator, in a chamber containing oxygen under pressure, and perfusing it with a balanced electrolyte solution containing neither cells nor haemoglobin.

An alternative approach to the problem is to try to freeze and thaw whole kidneys without loss of viability, using techniques similar to those developed by Parkes and his colleagues at the National Institute for Medical Research, London, for freezing and thawing whole animals (p. 172).

HOMOTRANSPLANTATION AND ISOTRANSPLANTATION OF THE KIDNEY IN PRESENT DAY SURGERY

Indications

Homotransplantation of a kidney is indicated in patients with severe chronic renal failure, and also in those with acute loss of, or irreparable damage to, both kidneys or a sole functioning kidney, under the following conditions.

1 If a healthy identical twin, possessing two normal kidneys, is available as a donor

2 If the patient has a non-identical twin fulfilling the other criteria enumerated above, and both the patient and his twin show the phenomenon of blood group chimerism and tolerate reciprocal skin homografts.

3. If the patient is suffering from congenital agammaglobulinaemia.

It should be possible by careful physical examination and blood grouping to reduce the chance of a non-identical twin being erroneously accepted as identical to perhaps one in ten, but as a general rule small test skin grafts should be interchanged before deciding to proceed with the transplantation of a kidney. The chance of a pair of non-identical twins showing blood group chimerism and associated mutual tolerance of each other's tissues is admittedly very small (p. 119), but may be a little greater than is commonly supposed, and the tests required to detect this state of affairs cause little inconvenience to either partner.

Is homotransplantation of a kidney indicated under any other circumstances? At present the chances of permanent survival are very small, although there is quite a good chance of a homotransplant functioning for a few weeks or even for a few months. Desperate situations, however, may justify desperate remedies, and when a patient—especially a young patient—has been deprived of a sole functioning kidney there are three possibilities worth considering: (a) transplantation of a kidney from the mother; (b) total body irradiation followed by homotransplantation of haemopoietic tissue together with a kidney from the same donor; (c) total body irradiation followed by homotransplantation of haemopoietic tissue from a foetus and a kidney from an adult donor.

Transplantation of a kidney from the mother is suggested in the light of the observation of Peer and his colleagues (p. 129)

that homografts of skin from mother to child sometimes survive for months or years

The rationale of the other procedures, and the prospects of success, have been discussed in *Chapter 22*.

It seems desirable, if the haemopoietic *tissue is obtained from an adult, to use the* same donor for the kidney, because otherwise the transplanted haemopoietic tissue may react immunologically against the kidney and destroy it Even so there is risk of a graft against host reaction, but if this is not too severe it may actually be advantageous, because it may further impair the homograft rejection mechanism of the recipient without doing him irreparable injury

If foetal haemopoietic tissue is used there is probably little danger of it reacting against either the host or a kidney from an adult donor, but it is as yet uncertain whether satisfactory haemopoiesis, and a satisfactory general immunological status, *will be restored*

It will be observed that no mention has been made of renal homotransplantation following total body irradiation without transplantation of haemopoietic tissue The reason is that it seems unlikely that this procedure will ever result in permanent survival of the transplant in the absence of some special relationship between donor and host

In the author's view it is not justifiable to use any of the above procedures unless the following conditions are fulfilled

1 The donor, and as a rule the patient also (or his parents if he is a child), are informed that the transplant may not function at all, and the chance of it functioning permanently is very small

2 The risks of the procedure are explained frankly to the patient (or his parents)

3 The patient (or his parents) and the donor are nevertheless anxious for the operation to be performed

4. The patient is thoroughly investigated before operation

5 Proper arrangements are made for follow up studies, and every effort will be made to secure an autopsy if the patient dies.

In cases of acute renal failure in which *there appears to be some prospect that the* kidneys will recover, homotransplantation offers a possible alternative to haemodialysis or irrigation of an ileal loop It does not seem justifiable to sacrifice a kidney from a living donor under these circumstances, but a cadaver kidney could reasonably be used *if one were available.*

Technique

When it is hoped that the transplant will survive permanently, cutaneous ureterostomy should be avoided, and probably the *best procedure is to place the kidney in the* pelvis, and anastomose the renal artery end-to end to the internal iliac artery, the renal vein end to side to the external iliac vein, and the ureter to the bladder, as described by Merrill *et al* and Murray *et al.* (p 535).

On the other hand, in patients with acute renal failure whose own kidneys seem likely to resume function in a week or two, cutaneous ureterostomy is permissible and indeed possibly advantageous, and transplantation to the groin according to the technique of Hume *et al* (p 530) would seem to be the operation of choice

It seems desirable that when an adult donor other than the patient's mother is used, *the donor and host should be com*patible at least in respect of the ABO and Rh blood grouping systems, and if possible of other systems also

In all cases the period for which the transplant is ischaemic should be kept to a minimum A reasonable target at which to aim *is 1 1½ hours, and 3½ hours should be re*garded as the absolute upper limit.

In our present state of knowledge the kidney should not be perfused after bein

moved from the donor. It should be kept cool but not frozen

Broad spectrum antibiotics should be administered routinely after the operation.

Haemodialysis (Kolff, 1947; Murphy, Swan, Walter, Weller and Merrill, 1952, Merrill, 1955; Parsons and McCracken, 1957) may be used both pre-operatively and post-operatively if the patient has symptoms of uraemia and does not respond to simpler measures. It is particularly likely to be required after operation when the transplant was subjected to a prolonged period of ischaemia.

FUTURE PROSPECTS

It seems possible, in the light of developments in the conservative surgery of the kidney, that it might be of value in some circumstances to remove a kidney temporarily, operate on it "on the bench," and replace it as an autotransplant. So far however there does not appear to be any record of this having been attempted

The widespread use of renal homotransplants to provide permanent replacement must await a solution, or at least a partial solution, of the biological homograft problem, and the development of satisfactory methods of storage Long-term storage is of course desirable, but a reliable method of storing kidneys for even a few hours would be enormously valuable.

In the author's view the avenues of research which seem likely to prove most profitable are as follows:

1 Autotransplantation of the kidney in experimental animals with the three-fold object of (a) improving technique—especially methods of dealing with the ureter, (b) learning more about the effects of permanent denervation of the kidney, and (c) assessing the value of the different methods of storage.

2. Further investigation of homotransplant immunity using free grafts of skin and other tissues.

3. Investigation of the difference in behaviour of homotransplants of whole organs by vascular anastomosis and free homotransplants.

4. Observations on the behaviour of renal homotransplants in man when their use is justified in accordance with the conditions discussed in the preceding section.

CHAPTER 26

Transplantation of the Lung, Heart, Spleen and Liver

In this chapter we shall consider some further *instances of transplantation of* viscera by vascular anastomosis At present none of them come within the scope of accepted clinical practice, but they are of importance because they point the way to possible future developments

Most of the work to be described has been concerned with homotransplants As in the case of the kidney, however, it would, in the author s view, be advantageous at the present time to use autotransplants of the organs in question to study the problems of operative technique and storage, and the capacity of the organs to function when deprived of their nerve supply It is not suggested that experiments with organ homotransplants should be abandoned, but they should be planned as far as possible in the light of information obtained with autotransplants, and should be used primarily to check the general validity of discoveries made with simpler types of homotransplant, including in particular free homotransplants of skin

THE LUNG

Transplantation of the Lung in Animals

Juvenelle, Citret, Wiles and Stewart (1951), after several unsuccessful attempts, succeeded in replacing the lung of a dog as an *autotransplant* after pneumonectomy The operation was performed in May, 1950 The right pleural cavity was opened under positive pressure intratracheal anaesthesia, and the azygos vein was clamped, divided and ligated to facilitate exposure of the lung The right main bronchus was cleared and divided, and the proximal stump was closed temporarily with Allis forceps The pulmonary artery and the two pulmonary veins were then cleared—the artery up to the point of division of the main pulmonary trunk and the veins to their entrance to the left auricle—and divided between clamps The vessels and bronchus were reconstituted by end to end anastomosis with silk Interrupted sutures were used in the bronchus, and while they were being inserted the opposite lung was kept inflated by introducing oxygen and ether vapour through a tube passed through the stump of the right bronchus and on into the left main bronchus The animal survived, and six months later was alive and well No abnormality was found on clinical or radiological examination of the chest Angiocardiography, performed 18 days after operation, also showed no abnormality

Métras (1950) studied the behaviour of lung homotransplants* in dogs He conserved part of the left auricle, *including* the openings of the two pulmonary veins, with the transplant, and anastomosed this to the left auricle of the host, thus avoiding

*Homotransplants that is according to the terminology adhered to throughout this book. Métras actually used the term hétérogreffe

two difficult venous anastomoses. He made end-to-end anastomoses of the pulmonary artery and of the main bronchus, and in addition connected the bronchial artery of the transplant to the subclavian artery of the host by a procedure similar to the "patch method" described by Carrel and Guthrie (1906c). He found that the transplanted lungs functioned for about 8 days, and the hosts survived for 20 to 28 days.

Staudacher, Bellinazzo and Pulin (1950) experimented with autotransplants and homotransplants of pulmonary lobes, but they used a non-suture form of anastomosis and their experiments were not very successful. Davis, O'Connor, Colovirás and Strawn (1952) performed homotransplantation of the lung in five dogs, but four of them died within 3 days of operation, partly owing to the large doses of heparin they received.

Neptune, Weller and Bailey (1953), also working with dogs, made a successful autotransplant by a technique very similar to that of Métras except that it did not include anastomosis of the bronchial artery, and in addition studied 25 homotransplants.

The animal in which autotransplantation was performed was alive and well more than a year after operation. The transplant appeared to be functioning well, and auscultation, bronchoscopy and differential broncho-spirometry revealed no abnormality. A radiograph of the chest showed a slight shift of the mediastinum to the operated side but was otherwise normal.

Four of the animals which received homotransplants were given ACTH (20 mg. daily in divided doses), and the average period of survival (excluding one animal whose transplant was removed 42 days after operation) was 25 days. The average period of survival of the other animals, which received no ACTH, was 6 days.

The day after operation the animals which received homotransplants ate and drank, and would walk on leash. They continued to appear healthy for about half of the survival period, and during this time radiography showed that the transplant was aerated and bronchoscopy showed that the bronchus was patent. Thereafter the animals became listless and refused to eat, auscultation and radiography showed that the transplant was no longer aerated, and bronchoscopy usually showed bloody necrotic debris in the bronchus and evidence of extrinsic compression beyond the suture line. At autopsy the transplanted lung appeared grossly enlarged, dark red and solid. There was often thrombosis in the smaller pulmonary veins but rarely in the pulmonary artery. Histological examination showed consolidation and necrosis, with accumulation of erythrocytes and polymorphonuclear leucocytes in the alveoli. The host's own lung was usually normal but occasionally showed small areas of consolidation.* In a few animals a slough of the bronchial anastomotic area was observed and it was thought that this might be due to loss of the bronchial circulation, which according to Ellis, Grindlay and Edwards (1951) is necessary for the nutrition of the main bronchi in dogs. This question has recently been investigated further by Bogardus (1958), who found that dogs in which the bronchial artery had been ligated on one side seldom survived removal of the opposite lung, and concluded that ligation of the bronchial artery seriously impairs the function of the lung.

Further experiments with lung transplants have been performed in dogs by Ellis and Richards (1951), Hughes, Kehne and Fox (1954), Lanari, Croxatto and Molins

*In two instances the behaviour of the transplants was atypical. One animal which received no ACTH developed distemper and died 27 days after operation while the transplant was still aerated. In another animal, which received ACTH, the transplant did not become atelectatic until 31 days after operation, when the bronchus was found to be blocked with granulation tissue. Attempts to remove the obstruction failed but the animal recovered when the whole transplant was removed 11 days later.

(1955 1956) and Hardin (1956a), and in sheep by Borrie and Montgomerie (1958)

Ellis and Richards (1954) removed the left lung and replaced it by the left lung of another dog The bronchus was anastomosed first, then the main pulmonary artery and finally the inferior and superior pulmonary veins The ten recipients survived for periods ranging from 21 hours to 14 days the mean being 6 days In the animals which lived for more than 3 days normal breath sounds were heard on auscultation up to within 18 hours of death At autopsy in the animals which died soon after operation the transplant was aerated and showed little evidence of inflammation In the animals which survived longer on the other hand the transplant was wet heavy and airless, its cut surface was red and firm and histological examination revealed necrosis of the alveolar walls and complete loss of the normal architecture of the lung All the vascular anastomoses were intact and an angiogram of the excised lung showed that the major vessels were patent but injection failed to demonstrate patency of the smaller branches of the pulmonary artery

Hughes *et al* (1954) removed the left lung and replaced it by the left lower lobe from the same or another dog The autotransplants appeared to function satisfactorily whereas most of the animals which received homotransplants died within a few days

Lanari *et al* (1955 1956) in more extensive experiments confirmed that a pulmonary lobe transplanted autologously functioned indefinitely despite the loss of the bronchial arterial supply and they attributed this to the existence of precapillary anastomoses between the bronchial and pulmonary arterial systems Homotransplants appeared indistinguishable from autotransplants for a few days but thereafter showed characteristic histological changes including congestion oedema thrombosis perivascular round cell infiltration and eventually necrosis and ceased to function after 7 10 days

Hardin (1956a) as we saw in Chapter 5 (p 74) found that skin homografts were rapidly destroyed in dogs which had previously received a lung homotransplant from the same donor and *vice versa*, thus showing that lung and skin have transplantation antigens in common

Borrie and Montgomerie (1958) developed a technique for autotransplantation of the left lung in sheep in which they anastomosed the common pulmonary venous trunk to the left auricle None of their animals lived longer than 6 days Some died within 24 hours of operation as a result of tension pneumothorax haemorrhage or bronchial obstruction Later deaths were attributed to thrombosis in the pulmonary artery or veins causing pulmonary infarction

It seems clear that homotransplants of lung evoke the same sort of reaction as homotransplants of most other tissues and organs and suffer the same fate Autotransplants behave very differently but it is not yet clear whether some of the failures are due to loss of the bronchial arterial supply rather than to technical errors It also remains to be determined whether the denervation which inevitably occurs has any significant effect on the function of an autotransplant

Experiments have also been performed in which the heart and both lungs have been transplanted *en bloc*, but these will be considered later (p 548)

The Outlook for Transplantation of the Lung in Surgery

There is no record of transplantation of a lung ever having been performed in man, and at present there is no indication for attempting such an operation

The idea of removing a lung operating on it on the bench and then replacing it as

an autotransplant, seems far-fetched but might one day prove useful. It would, however, have to be shown by more extensive experiments in animals, and if these were successful by cautious clinical trial, that such a replaced lung was capable of functioning effectively for a long time, under conditions of stress as well as of rest. Whether or not it would be necessary to re-establish circulation through the bronchial artery in man remains to be seen.

Homotransplantation would certainly be of value in some patients with seriously impaired pulmonary function if permanent survival could be achieved; at present, however, this could be done only by using an identical twin as donor. In the author's view it would be unjustifiable at present to remove a normal lung from a living donor for transplantation, and the chance of obtaining one *post mortem* from an identical twin at the appropriate time is very remote.

Use of the Perfused Lung as an Oxygenator

It may be mentioned that a perfused lung has been used successfully as the oxygenator in cardio pulmonary bypass (p. 125).

Potts, Riker, DeBord and Andrews (1951, 1952) reported successful acute experiments in dogs in which a homologous lung was used for this purpose. Mustard and Chute (1951) obtained long term survival in dogs after clamping the superior and inferior venae cavae and maintaining the animals with a mechanical heart and homologous lung oxygenator for periods up to 17 minutes, and subsequently this time was extended to 42 minutes by Mustard, Chute and Simmons (1952), and to 45 minutes by Fischer, Albert, Riker and Potts (1952).

A similar system using a heterologous lung has been used to maintain the circulation in human patients during operations on the heart. Mustard, Chute, Keith, Sirek, Rowe and Vlad (1951) reported seven patients with transposition of the great vessels in whom an attempt was made to correct the condition surgically while the circulation was maintained with a mechanical pump and monkey lung oxygenator. None of the patients survived, but it was felt that this form of extracorporeal circulation should be tried in less hopeless cases. Varco (personal communication) has had encouraging results using a dog lung as oxygenator.

It appeared at one time that the perfused lung might prove in the long run to be more satisfactory than mechanical oxygenators. It is certainly effective, and such evidence as there is suggests that it causes relatively little trauma to the blood constituents. In addition, as Mustard *et al.* (1952) have pointed out, the perfused lung is an efficient filter and is able to perform "certain detoxification functions which are not [yet] clearly understood." There are however some serious disadvantages. Heterologous lung is readily available but problems of sensitization might conceivably arise even after perfusion for only an hour or two, while fresh human lung is unlikely to be available when required and so far no satisfactory method of storage has been devised. It is fortunate therefore that mechanical oxygenators have now been developed to such an extent that for clinical purposes the use of a perfused lung need no longer be considered.

THE HEART

The first experiments on transplantation of the mammalian heart were concerned with heterotopic homotransplantation. More recently, with the introduction of hypothermia and artificial pump-oxygenators, it has become possible to transplant the

heart orthotopically in experimental animals, but this procedure has not been attempted in man

Heterotopic Homotransplantation of the Heart

Mann, Priestley, Markowitz and Yater (1933) developed a technique for transplanting the heart from a puppy to the neck of an adult dog They anastomosed the aorta of the transplant, or one of its main branches, to either the proximal or the distal end of the divided left carotid artery of the host, and the left pulmonary artery of the transplant to the proximal end of the divided right external jugular vein of the host (Fig 165) By this procedure the coronary circulation was re established and, if the operation was correctly performed, the heart began to beat in its new site and continued to do so regularly for a period ranging from one to eight days Pulsation then ceased, *either suddenly and unexpectedly or* after a period of irregular rhythm and in some cases fibrillation

The main causes of operative failure were distension of the heart with blood before the beat was re established, undue delay in re establishing the coronary circulation, and air embolism in the coronary vessels These were avoided by (a) a judicious choice of the order in which the vessels were tied while removing the heart from the donor,* (b) reducing the period during which the transplant was deprived of a coronary circulation to less than five minutes by preparing the vessels in the host before removing the heart from the donor, and by operating quickly, (c) temporarily opening one vena cava before the heart beat was re established to allow escape of blood accumulated in the right auricle, and (d) allowing blood—and with it any air which had entered the vessel—to leak from the aortic anastomosis before removing the proximal clamp In

*Mann *et al* described in detail two alternative techniques which proved equally successful

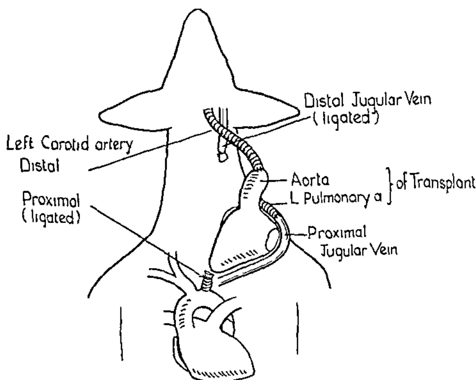


Fig 165 Method of connecting a transplanted heart used by Mann Priestley, Markowitz and Yater For description see text

some cases heparin was administered to the donor as an additional precaution against intravascular clotting. It made little difference whether or not the pericardium was retained with the transplant provided that if it was retained a large opening was made to allow escape of pericardial fluid.

When the transplanted heart was removed just before it ceased to beat the left auricle was found to be filled with clot, and the right auricle and ventricle were distended. The surface of the heart was covered with mottled areas of ecchymosis. The muscle was friable, and on histological examination was found to be infiltrated with round cells and polymorphs. Mann *et al.* concluded from the findings that the cessation of function was due "to some biologic factor which is probably identical to that which prevents survival of other homotransplanted tissues and organs".

Sinitsyn (1948, 1956), Downie (1953), Wesolowski and Fennessey (1953), and Sayegh and Creech (1957) performed experiments very similar to those of Mann *et al.* Sinitsyn* placed the transplants in either the neck, the thorax or the abdomen, and used celloidin cannulae in making his anastomoses. The transplants yielded a normal electrocardiogram for two or three days and survived for up to 30 days.

Downie, and also Wesolowski and Fennessey, placed the transplant in the neck. The beat was restored in all but a few cases and continued for up to 10 days (average 129 hours) in Downie's experiments and up to 7 days in those of Wesolowski. Downie reported that on histological examination the transplants showed only a slight degree of cellular infiltration, and argued from this that the cessation of function was brought about by humoral factors, but this conclusion is unjustified and almost certainly erroneous.

*Sinitsyn also performed transplantation of the heart in frogs but we shall consider only his experiments in dogs.

Sayegh and Creech placed the transplants in the neck, except in three instances when they were placed in the groin and anastomosed to the femoral vessels. Some of the transplants were obtained from new-born puppies and others from foetuses during the last two weeks of gestation. It had been hoped that they would evoke a less intense reaction than transplants from older donors and in consequence survive longer, but in fact no transplant continued to beat for more than 10 days, and the average duration of activity was only 73 hours for the foetal hearts and 81 hours for the puppy hearts.

Marcus, Wong and Luisada (1951, 1953) introduced a procedure termed *interim parabiotic perfusion* in which, by using a third dog as blood donor, they were able to maintain an uninterrupted circulation in the coronary vessels of the transplant throughout the whole operation.*

Three different patterns of vascular connexion between transplant and host were studied.

In the first, the innominate artery and aorta of the transplant were anastomosed to the proximal and distal ends respectively of the divided carotid, common iliac or femoral artery of the host, and the left pulmonary artery of the transplant was anastomosed to

*After anaesthetizing all three animals an intravenous saline infusion was started in the blood donor and cannulae were inserted into the proximal ends of the divided carotid artery and external jugular vein. The heart donor was then prepared by performing bilateral cervical vagotomy, opening the chest under positive pressure intratracheal anaesthesia, and injecting 2-4 per cent cocaine into the pericardial sac. Cannulae were inserted into the left subclavian artery and superior vena cava of this animal, and connected by tubing with the cannulae in the blood donor's carotid artery and jugular vein respectively. The vena azygos, inferior vena cava, aorta and remaining aortic branches were clamped and the clamps on the perfusion tubing were released. The heart and lungs were removed *en bloc* and placed in Ringer's solution at 38°C. The lungs were then removed, and the heart, which was still beating, was transplanted to the host, or alternatively, the heart and lungs were transplanted together. The perfusion was terminated when the vascular anastomoses between the transplant and the recipient had been completed.

and returned via a pump oxygenator to the femoral artery If homotransplantation is being undertaken it is convenient to have separate teams operating simultaneously on the donor and recipient The heart, alone or with one or both lungs, is removed from the recipient and replaced by the corresponding viscus or viscera from the donor, care being taken to preserve the phrenic nerves in the recipient The heart of the donor may be arrested by injection of potassium citrate The aortic anastomosis is performed first so that coronary flow is restored as soon as possible. Even so some delay is inevitable, and to minimize the effects of this some investigators advise cooling the heart and also perfusing the coronaries with Ringer's solution (Webb and Howard, 1958)

Webb and Howard (1958) performed autotransplantation and homotransplantation of the heart and both lungs, and also homotransplantation of the heart and left lung They found that when both lungs were transplanted with the heart the recipients never breathed spontaneously, and they attributed this to the fact that both lungs were denervated When the recipient retained one of his own lungs intact, on the other hand, spontaneous respiration was temporarily restored as soon as the chest was closed and artificial respiration discontinued

Blanco, Adam, Rodriguez Perez and Fernandez (1958) performed homotransplantation of the heart and both lungs on eight occasions. In six of the recipients the transplanted heart began to beat and maintained the systemic circulation for periods ranging from ½ to 1½ hours (mean 2½ hours) after the pump oxygenator had been disconnected Contrary to the experience of Webb and Howard spontaneous respiration returned in two animals The cause of death in every case was ventricular fibrillation

Berman, Goldberg and Akman (1958) performed homotransplantation of the heart alone in six dogs To avoid the difficult task of anastomosing the pulmonary veins they divided the left auricle central to the orifices of these veins in both donor and recipient and anastomosed auricle to auricle The operation was completed successfully in five animals and the transplanted heart functioned for periods ranging from 21 minutes to 2 hours In two of these animals ventricular fibrillation was terminated by applying a defibrillator or arresting the heart with potassium, and normal sinus rhythm was temporarily restored

It seems clear that the transplanted—and hence denervated—heart can maintain the circulation for a few hours, but whether it can do so for longer periods remains to be seen Further studies with autotransplants are urgently needed to settle this question

THE SPLEEN

There do not appear to have been any experiments on transplantation of the spleen by vascular anastomosis in animals,* and as the spleen is not essential for life there would seem at first sight to be no indication for transplanting this organ in clinical practice On closer consideration, however, it seems just possible that homotransplantation of the spleen may prove to be of value in one particular condition, *agammaglobulinaemia* Patients suffering from this condition are subject to recurrent severe infection and, as we have noted (p 64), have been reported to be excep

*Free transplantation of small pieces of spleen has been used by many investigators in studying homotransplant immunity (Chapters 3 7) Free autotransplantation in the procedure designed by Cameron and De Saram (1939) for permanently dissociating the spleen from the portal circulation in experimental animals

tionally tolerant of homografts of skin. In the acquired form of the disease the tolerance is only partial, but in the congenital form it appears to be virtually complete. It is conceivable therefore that a homotransplant of spleen or bone marrow might survive permanently, and if so might provide the patient with a permanent antibody-forming mechanism. On the other hand the transplant might cause a serious graft against host reaction (p. 457), or alternatively it might survive for a time but later bring about its own destruction by enabling the patient to react in a normal way to homotransplants in general, and to itself in particular.

To investigate the matter Nicks (personal communication) transplanted the entire spleen from an infant who died two days after birth to a man aged 21 with a long history of recurrent infection who appeared from paper electrophoresis studies to be agammaglobulinaemic. The spleen was placed deep to the rectus abdominis muscle, and the splenic artery and vein were anastomosed to the inferior epigastric artery and vein respectively. When the clamps were released the transplant increased in size, became pink in colour and showed rhythmic contraction.

After the operation the electrophoretic pattern of the plasma proteins remained unchanged, but it is doubtful whether any significance can be attached to this because no evidence has been adduced to show that the vascular anastomoses remained patent. The investigation is, moreover, open to criticism on several other grounds. In the first place the patient appears to have been suffering from the acquired form of agammaglobulinaemia, and is therefore unlikely to have been completely tolerant of homografts. Secondly, paper electrophoresis is insufficiently accurate for a study of this kind. Thirdly, an unnecessary complication was introduced by making small free transplants of skin and liver tissue from the same donor simultaneously with the spleen transplant. Finally, the spleen, after being removed from the donor, was perfused with heparinized Darrow's solution, and judging from experiments on transplantation of the kidney (p. 515), this was almost certainly not beneficial and may have been harmful.

In the author's view it is not justifiable at present to perform further operations of this kind owing to the risk of a graft against host reaction (*v. supra*) should the transplant survive.

THE LIVER*

Goodrich, Welch, Nelson, Beecher and Welch (1956) have devised a technique for homotransplantation of the dog's liver in which the transplant is located in the abdomen below the host's own liver, which is not removed (Fig. 167). The donor's liver is exposed through a thoraco-abdominal incision by one operating team. At the same time another team opens the abdomen of the recipient, divides the inferior vena cava below the renal vessels between clamps and inserts a Blakemore-Lord cuff (p. 418) in the cranial end, and divides the aorta between clamps and inserts a polyethylene tube into this vessel above the level of division. When the recipient has been prepared in this way the first team divides, in order, in the donor the infrahepatic vena cava, the portal vein, the aorta below the superior mesenteric artery and above the coeliac axis, the superior mesenteric artery and the branches

*We are concerned primarily with transplantation of the liver by vascular anastomosis, but it may be mentioned that Seneviratne (1955) has developed a technique for pedicle autotransplantation of the median lobe of the rat liver into the abdominal wall which has proved useful in studying regeneration of the liver.

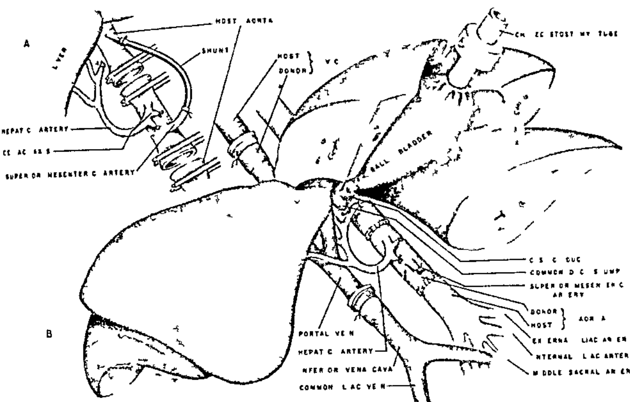


Fig 167 Diagram illustrating the technique of homotransplantation of the canine liver devised by Goodrich Welch Nelson Beecher and Welch (Reproduced from *Surgery* by courtesy of the publisher and Dr G Stuart Welch)

of the coeliac axis other than the hepatic artery and the suprahepatic vena cava ligating the infrahepatic vena cava and the divided branches of the coeliac axis but not the other vessels The common bile duct is ligated and divided and the liver is then placed in the abdomen of the recipient and the suprahepatic vena cava of the transplant is anastomosed to the vena cava of the host using the previously placed Blakemore Lord cuff The polyethylene tube leading from the aorta of the recipient is connected to another tube inserted into the stump of the superior mesenteric artery of the transplant and with this shunt in position the proximal and distal aortic anastomoses can be performed accurately and without hurry When these anastomoses have been completed the shunt is discontinued and the portal vein of the transplant is connected to the recipient s distal vena cava over a second Blakemore Lord cuff The diaphragmatic tags on the suprahepatic vena cava of the transplant are sutured to the fascia over the right psoas muscle and a cannula is inserted into the gall bladder of the transplant

Goodrich *et al* performed this operation successfully nine times in thirteen attempts They also transplanted the liver without using a temporary arterial shunt after administering wide spectrum antibiotics to eliminate as far as possible the clostridia which are present in the dog s liver and proliferate rapidly under anaerobic conditions, but were successful only twelve times in thirty six attempts

When the operation was successful the transplant began to secrete bile almost immediately The output increased to a maximum about the third day and usually ceased after five days Signs of toxaemia then appeared sanguino purulent material

began to be discharged through the cannula in the gall bladder, and soon afterwards the animal died.

The histological findings varied but generally speaking in successful transplants round cell infiltration of the periportal spaces began about the fourth day. This was followed almost immediately by necrosis, which began in the centre of the lobules but progressed rapidly and usually involved the whole liver by the end of the fifth day

Cannon (1956) has attempted to perform orthotopic homotransplantation of the liver in dogs under mild hypothermia after removing the host's own liver, but so far without success

Torrance (unpublished), in the author's laboratory, attempted in two acute experiments to transplant the left lobe of the canine liver into the thoracic cavity, the left subclavian artery being anastomosed to the cut end of the portal vein and the hepatic veins to the lateral surface of the left atrium. On removal of the clamps the liver became pink and the circulation in the transplanted lobe appeared to be adequate If this technique is to be used in survival experiments some means of collecting bile from the transplant will have to be provided, for example, in the form of a Roux loop of jejunum (p 561)

The human liver normally contains few micro-organisms, and in this respect is a more favourable organ for transplantation than the dog liver. In view of the technical difficulty of the operation, however, and the even greater difficulty of obtaining suitable donor material, it would be a most formidable undertaking to attempt to replace the liver of a patient by a homotransplant even if the biological homograft problem were completely solved. It is conceivable however, as Goodrich *et al.* have suggested, that a temporary liver homotransplant might be of value in treating patients with acute but not irreversible liver failure.

Another approach to the problem of treating reversible liver failure is suggested by the observation of Otto, Pender, Cleary, Sensenig and Welch (1958) that the blood ammonia in animals with experimental ammonia intoxication* can be reduced by perfusing blood from the intoxicated animal through a homologous liver and then returning it to the circulation. It would however be almost impossible to obtain a sufficiently fresh human liver for clinical use, and the possibility of sensitization and other complications is enough to discourage attempts to use heterologous liver, at any rate until it has been thoroughly tested in experimental animals.

*Produced by performing porto caval anastomosis and then administering ammonium chloride or putting the animal on a high protein diet

CHAPTER 27

Pedicle Transplantation and Anastomosis of Hollow Viscera
I. Reconstruction of the Alimentary Tract

In this and the following chapter we shall *consider some examples of pedicle* transplantation and anastomosis of hollow viscera undertaken for the most part to relieve obstruction, *to* restore continuity after resection,* or to provide a reservoir

TRANSPLANTATION OF STOMACH, JEJUNUM AND COLON TO THE OESOPHAGUS AND PHARYNX

In dogs, continuity of the thoracic oesophagus† has been restored successfully by end to end anastomosis after resection of segments ranging in length from 3 to 7 cm (Dobromysslow, 1901, Sauerbruch, 1905, Zaaijer, 1911, Omi and Karasawa, 1913 Enderlen, Hotz and Porzelt, 1914, Swenson and Clatworthy, 1947, Parker and Brockington 1949) *Indeed, in a later* paper *than the* one cited above, Swenson and Clatworthy (1949) reported that half the total length of the thoracic oesophagus could always be resected safely and that some dogs survived after resection of even greater lengths and end to-end anastomosis It has been shown further that the whole length of the thoracic oesophagus can be mobilized without gangrene resulting (MacManus Dameron and Payne, 1950)

In man, resection and end to end oesophageal anastomosis was first performed successfully by Haight and Towsley (1943) in an infant with congenital atresia of the oesophagus and oesophago tracheal fistula Subsequently many other successful cases have been reported (Haight 1944 Ladd 1944 Gross and Scott 1946 Ladd and Swenson 1947 Swenson 1947a Franklin 1947 1948 Bigger 1949 Potts, 1950a Gross 1953, and others) and it is now generally *agreed that resection and end to end anastomosis* is feasible and is the procedure of choice in the great majority of these infants

There are a few recorded cases in which oesophageal resection and anastomosis has been performed successfully in older patients for a simple stricture (Swenson and Clatworthy, 1947, Gross, 1948 Weisel Pink and Fitzsimmons 1955) or as a palliative operation for incurable carcinoma (Parker and Brockington, 1949) and the oesophagus has also been successfully anastomosed to the pharynx after pharyngectomy (Wooler, 1952) and after resection of a stricture of the cervical oesophagus (Weisel, Pink and Fitzsimmons 1955) most surgeons however, have been deterred by the fear that mobilization sufficient to permit approximation of the ends without undue tension would re

*We shall not consider restoration of continuity by direct anastomosis of the free ends when the environmental change which results is of a minor kind

†As far back as 1872 Billroth resected about 1.5 cm from the cervical part of a dog s oesophagus and restored continuity successfully by end to end anastomosis

sult in avascular necrosis (cf. Sweet, 1946).

While these fears may have been exaggerated it is clear that in many cases restoration of continuity of the oesophagus in adults by direct anastomosis is impossible, and various techniques have been devised to deal with this situation. One way of proceeding is to create an oesophageal fistula above and a gastric fistula below, and to join them by a removable rubber tube or by a tube of skin fashioned on the front of the chest. Despite some brilliant successes, first by Franz Torek* (1913, 1925) and subsequently by Hedblom (1922), Braizew (1929), Grey Turner (1933), King (1936), Yudin (1944) and others, these methods are now obsolete.† The rubber tube was messy and inconvenient, and skin tubes, as we have seen (p. 265), proved unsatisfactory because of the frequency with which fistulae developed, especially at the lower junction. The alternatives, which will now be considered in detail, are pedicle transplantation of part or all of the stomach, and pedicle transplantation of a segment of jejunum or colon.

TRANSPLANTATION OF THE STOMACH

History

Three types of procedure have been used:

1. Antethoracic transplantation of a tube constructed from the greater curvature or anterior wall of the stomach.
2. Antethoracic transplantation of the stomach as a whole.
3. Intrathoracic transplantation of the stomach.

*In the same year that Torek performed his celebrated resection of the oesophagus Zaaijer (1913b) resected a carcinoma of the cardia by a thoraco-abdominal approach.

† Torek's operation, despite its defects, was the standard procedure for carcinoma of the middle third of the oesophagus until 1944, and as late as 1950 Shaw reported two cases in which antethoracic skin tubes were employed. Useful reviews of the earlier work have been published by Saint (1929), and Ochsner and Owens (1934).

Antethoracic Transplantation of a Tube Constructed from the Stomach

Beck and Carrel (1905) in America, and Jianu (1912) and Halpern (1913) in Germany, showed—apparently independently—in dogs that a tube constructed from the greater curvature of the stomach and nourished by the left gastro-epiploic artery could be brought up subcutaneously in front of the chest far enough to be anastomosed to the cervical oesophagus. They suggested, in the light of observations on cadavers, that the same procedure could be used in man. Hirsch (1911) suggested using a similar type of tube constructed not from the greater curvature but from the anterior wall of the stomach.

The Beck-Jianu type of oesophagoplasty appears to have been first used clinically by Ropke (1912), who modified the proposed technique however by making the tunnel for the gastric tube deep to the pectoral muscles. Soon afterwards Meyer (1913) reported three cases, in two of which the gastric tube was brought up *subcutaneously* as originally proposed.

Ochsner and Owens in 1934 found in the literature twenty-four cases, six with carcinoma and eighteen with benign stricture of the oesophagus, in which the Beck-Jianu or Halpern technique, or some slight modification, had been used, mainly by German, Austrian, Russian and Scandinavian surgeons. Five of the patients had died, and in all except two of the remainder the procedure had been abandoned before being completed. Jianu had hoped to obtain a tube from the stomach sufficiently long to be anastomosed directly to the oesophagus in the neck, but in most of the cases an intervening skin tube was used (cf. Lotheissen, 1913, 1922).

These operations were virtually abandoned when satisfactory methods of intrathoracic oesophagoplasty were established (p. 556), but have been used occasionally in recent times, for example by Poilleux and

Frileux (1950), who brought up a Jianu type of gastric tube antethoracically and anastomosed it to the cervical oesophagus as palliative treatment in a patient with an inoperable carcinoma

Antethoracic Transplantation of the Stomach as a Whole

Von Fink (1913) used the stomach as an antethoracic oesophagus in a patient with a carcinoma of the cardia He divided the duodenum, closed the distal end, mobilized the stomach except at the cardia, brought the duodenal end of the stomach upwards subcutaneously, and restored the continuity of the gastro intestinal tract by performing a posterior gastro enterostomy He completed the procedure by connecting the mobilized stomach to the cervical oesophagus by means of a skin tube, but unfortunately the patient died a few days later from perforation of his carcinoma Ochsner and Owens (1934) found six cases in the literature in which this procedure had been attempted, but in only three had it been completed

Kirschner (1920) transplanted the stomach antethoracically, with an isoperistaltic orientation, after dividing the cardia, and the left gastric, left gastro epiploic and short gastric arteries He anastomosed the lower end of the oesophagus to a loop of jejunum within the abdomen by means of a Murphy button, and eventually succeeded in one case in completing the procedure by making an anastomosis in the neck between the cervical oesophagus and the transplanted stomach

Ochsner and Owens (1934) found sixteen cases reported in the literature in which this or a similar technique had been used, but ten of them had died, and good function was obtained in only four

More recently Rienhoff (1948) and Potts (1950b) have transplanted the stomach antethoracically in infants with congenital atresia of the oesophagus in whom, after resection of the lesion, direct anastomosis of the oesophagus appeared impossible Rienhoff's two patients, who were operated on at the age of nine days and five days respectively both did well for a time but about a year later succumbed to infantile diarrhoea Potts patient was also operated on shortly after birth and the functional result was excellent The antethoracic stomach appeared so unsightly however that at the age of 22 months the stomach was retransplanted to an intrathoracic position

Intrathoracic Transplantation of the Stomach

Biondi (1895) in experiments in dogs, divided the lower thoracic oesophagus, pulled the stomach up into the chest and anastomosed it to the proximal oesophageal stump closed the distal stump and replaced the stomach in the peritoneal cavity so that the anastomosis was below the level of the diaphragm Despite this remarkable achievement little progress could be made in the direction of intrathoracic resection and reconstruction of the oesophagus until methods of maintaining respiration while the chest was open began to be developed during the first decade of the present century by Sauerbruch (1904), Brauer and Petersen (1904) Meyer (1909, 1910), Meltzer and Auer (1909), and other surgeons (for review *see* Green and Janeway, 1910)

The first experiments in which such methods were used appear to be those of Willie Meyer, and Janeway and Green

Meyer (1909) successfully resected the lower third of the oesophagus and performed oesophago gastrostomy in two dogs* out of six Through a left thoracotomy he mobilized the lower part of the oesophagus, divided it just above the diaphragm, and closed the distal stump He then incised the diaphragm, pulled a pouch of stomach up into the chest without attempting to mobilize the stomach as a whole, and made a su-

*One of these animals subsequently died of distemper

tured anastomosis between this pouch and the proximal stump of the oesophagus. Shortly afterwards (Meyer, 1910) he resected part of the oesophagus in a human patient with a carcinoma, but the lesion was too high for oesophago gastrostomy to be attempted During the operation the patient's head was in a positive pressure cabinet, and after being removed from the cabinet the patient became cyanosed and died in about ten minutes. Up to this time, according to Meyer, there had been thirty-eight intrathoracic operations for carcinoma of the oesophagus In twenty-one patients a resection was performed, and all of them died. In another patient, who also died, Sauerbruch had performed oesophagogastrostomy using a Murphy button as a short-circuiting operation without resecting the tumour.

Janeway and Green (1910), after preliminary experiments (Janeway and Green, 1909) in which they used the Murphy button technique of anastomosis, performed transthoracic resection of the lower oesophagus and sutured oesophagogastrostomy in dogs after extensively mobilizing the stomach and dividing both gastric and both gastro epiploic arteries, with success in ten animals out of seventeen They performed a similar operation, using a combined abdominal and thoracic approach, in a human patient, but he died two days later.

Further instances of intrathoracic oesophagogastrostomy in dogs were reported by various investigators including Omi and Karasawa (1913) and Miller and Andrus (1923). Meyer (1913) suggested anastomosing the oesophagus to a Beck-Jianu gastrostomy within the chest and in 1911 Jianu himself reported two patients with oesophageal strictures successfully treated in this way, but it was not until 1933 that a Japanese surgeon, Ohsawa, reported the first clinical cases of successful intrathoracic oesophagogastrostomy in which the stomach as a whole was transplanted. Ohsawa's work attracted little attention in Britain and America for some years, but his achievement was remarkable. He resected the cardia and performed oesophagogastrostomy as a one-stage procedure by a combined abdominal and thoracic approach in eighteen patients, and of these eight survived the operation.

Five years later Adams, Escudero, Aronsohn and Shaw (1938) in Chicago reported experiments in dogs in which one-stage resection of the lower oesophagus and oesophagogastrostomy was performed successfully by a transthoracic approach, and in the same year Adams and Phemister (1938) reported that they had performed a similar operation successfully in a patient with a carcinoma of the lower third of the oesophagus. In this case the left pleural cavity was opened and the stomach was exposed through an incision in the diaphragm from the oesophageal hiatus to the costal margin and mobilized, the left gastric artery being divided. The oesophagus was divided at the cardia and the opening in the stomach was closed. The growth was resected with a margin of healthy oesophagus, and the fundus of the stomach was brought up into the chest and anastomosed to the stump of the oesophagus Gastrostomy was performed but it was felt that this might be omitted in future cases. Further operations of the same kind were soon reported by Churchill and Sweet (1942), Phemister (1943), Adams (1945), Sweet (1945, 1948a), Thompson (1945), Tanner (1946) and many others.

Extension of this type of procedure to the treatment of carcinoma of the middle part of the oesophagus, for a time erroneously regarded as impossible (cf. Churchill and Sweet, 1942), was reported first by Garlock (1944), and subsequently by Sweet (1945, 1946), Clark (1945), DeBakey and Ochsner (1948), and others. Garlock, in his first case, divided only the upper two branches of the left gastric artery, the vasa brevia, and branches of the left gastro-epiploic artery to

the middle of the greater curvature, yet was able to bring the fundus of the stomach sufficiently high to permit anastomosis to the oesophagus above the level of the aortic arch To overcome the difficulty of mobilizing a tumour deep to the arch, Lewis (1946) and Tanner (1946), in Britain, introduced a different approach combining a right thoracotomy with laparotomy to permit mobilization of the stomach * Lewis initially advocated a two stage procedure whereas Tanner preferred to complete the operation in one stage.

A further advance was made when Sweet (1948a) and Garlock (1948) showed that it was feasible, after resecting tumours in the upper part of the thoracic oesophagus, to mobilize the stomach sufficiently to bring it up via the chest and anastomose the fundus to the cervical oesophagus in the neck Sweet found it convenient to resect the inner half of the clavicle and part of the first rib, Garlock, in his first patient,† did not resect any bone Sweet (1948b) also performed intracervical oesophagogastrostomy after pulling the stomach up through the chest in an infant aged 21 months with congenital atresia of the oesophagus in whom it had not been possible to restore continuity of the oesophagus by end to end anastomosis at the original operation, and which had been fed ever since through a gastrostomy Potts (1950b), having treated a new born infant with congenital oesophageal atresia, in whom oesophageal anastomosis was impossible, by transplanting the stomach antethoracically, re transplanted the stomach twenty two months later to an intrathoracic position and re anastomosed it to the oesophagus The child eventually recovered but showed great respiratory distress in the immediate post-operative period, apparently because of the amount of space in the chest occupied by the stomach, and it seemed clear that it would be highly dangerous to attempt this procedure at an earlier age It has now been shown (Gross, 1953), however, that a more limited transplantation of the stomach, just sufficient to enable end-to end anastomosis of the oesophagus to be performed when mobilization of the oesophagus alone has proved inadequate, can be performed as a primary procedure without undue risk

A gastric tube formed from the greater curvature of the stomach and pedicled on the cardia was transferred to the chest and anastomosed to the proximal end of the oesophagus by Heimlich (Heimlich and Winfield, 1955, Heimlich, 1957) A similar method has been used with conspicuous success by Gavriliu of Rumania (Gavriliu and Georgescu, 1951, 1955) to restore continuity after oesophagectomy for benign stricture or carcinoma These tubes can be readily drawn up into the neck and anastomosed to the cervical oesophagus

*The approach to the oesophagus from the right side had been suggested as long ago as 1917 by Hofer and Kofler It was used in three cases by Wookey in 1940, who demonstrated that the azygos vein could safely be divided Wookey did not open the abdomen (except at a previous operation to establish a gastrostomy or jejunostomy) because he used an antethoracic skin tube as a substitute for the oesophagus

†This patient died the day after operation His death was attributed to physiological disturbance caused by vagal stimulation

Use in Present Day Surgery

Antethoracic Transplantation

Antethoracic transplantation of tubes constructed from the stomach, and of the stomach as a whole, though still occasionally performed, should be regarded as obsolete

Intrathoracic Transplantation

Intrathoracic transplantation of the stomach and oesophagogastrostomy is the standard method of restoring continuity of the alimentary tract after resecting tumours of the oesophagus up to the level of the aortic arch It is used also, as an alternative to *oesophagojejunostomy*, to restore continuity after resecting benign strictures which have not responded to dilatation, and to

short-circuit inoperable tumours of the oesophagus and cardia. Access to lesions in the lower third of the oesophagus is by left thoracotomy, or by a left thoraco-abdominal approach. For tumours in the middle portion of the oesophagus the access may be from either side. Mobilization of the tumour is much easier from the right side because the vena azygos can safely be divided, but a separate abdominal incision is needed to mobilize the stomach because access through the diaphragm is impeded by the liver. The left gastric, left gastro-epiploic and short gastric vessels, and descending vessels in the great omentum, can safely be ligated and divided, but the right gastric and right gastro-epiploic vessels, and the vascular arcades along the greater and lesser curvatures, are carefully preserved. Mobilization is facilitated by removing the spleen, and many surgeons do this as a routine. The stomach is divided and closed at the cardia, and the oesophagus is anastomosed to the fundus with two layers of sutures (Fig. 168).

Transthoracic transplantation of the stomach and cervical oesophagogastrostomy is one of the methods used to restore continuity after resecting tumours of the thoracic oesophagus above the level of the

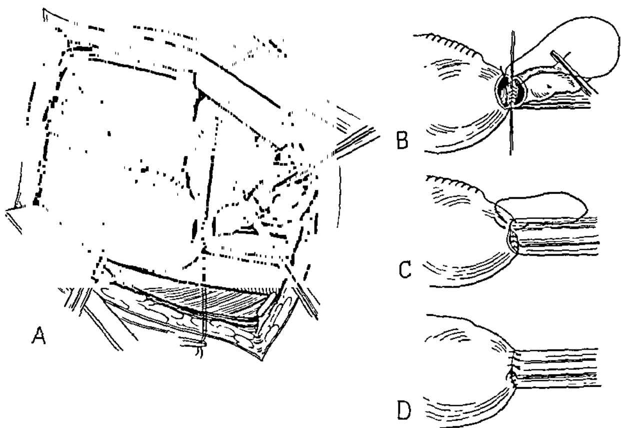


Fig 168 Reconstruction of the alimentary tract by oesophagogastrostomy after resection of a carcinoma of the lower third of the oesophagus A The stomach and lower oesophagus are mobilized. The oesophagus is divided at the cardia and the stomach is closed The stomach is brought up into the chest and a posterior row of interrupted sutures is inserted uniting the muscle of the oesophagus to the serous and muscular coats of the stomach The oesophagus and stomach are opened transversely B, C. A continuous all coats suture is inserted posteriorly, and continued anteriorly as the oesophagus is divided proximal to the tumour D Interrupted sutures are inserted anteriorly uniting the muscle of the oesophagus to the serous and muscular coats of the stomach Before the chest is closed the stomach is anchored to the mediastinal pleura by interrupted sutures

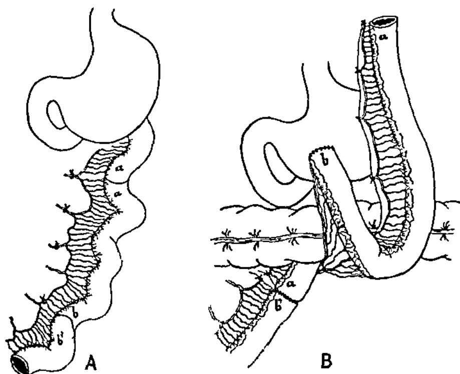


Fig 169 Roux's method of fashioning an isolated segment of jejunum to replace the oesophagus A Several jejunal arteries have been divided The bowel itself will be divided at aa¹ and bb¹ B The loop has been isolated and continuity of the bowel has been restored by anastomosing a to b¹ (Reproduced from *La Semaine Medicale* (1907))

aortic arch The procedure is a formidable one and the results are not very encouraging

Intrathoracic transplantation of the stomach to facilitate end to end anastomosis of the oesophagus is probably the procedure of choice in infants with congenital oesophageal atresia when mobilization of the oesophagus alone does not permit safe anastomosis * The operative approach in congenital atresia is from the right side but there is still some difference of opinion as to whether it should be extrapleural or transpleural The transpleural approach which is now preferred by the majority of surgeons permits more thorough mobilization of the oesophagus in addition it permits mobilization of the stomach† should this be required

TRANSPLANTATION OF JEJUNUM

History

Antethoracic Transplantation

Transplantation of a segment of jejunum to provide a substitute or partial substitute for the oesophagus was suggested by Wullstein (1904)‡ in the light of observations on the cadaver but the first operation of this kind was performed three years later by Roux (1907) in a child suffering from a benign stricture of the oesophagus Roux's

*The alternative is to ligate and divide the tracheo oesophageal fistula if present and establish a cervical *oesophagostomy and a gastrostomy Continuity may be* restored two or three years later by gastro or jejuno or colo oesophagoplasty

†That is to say mobilization sufficient to enable end to end anastomosis of the oesophagus to be performed As we have seen it is not feasible by a right transpleural and transdiaphragmatic approach to mobilize the stomach sufficiently to permit high oesophagogastrostomy after resecting a carcinoma in an adult

‡*See also* Wullstein (1908)

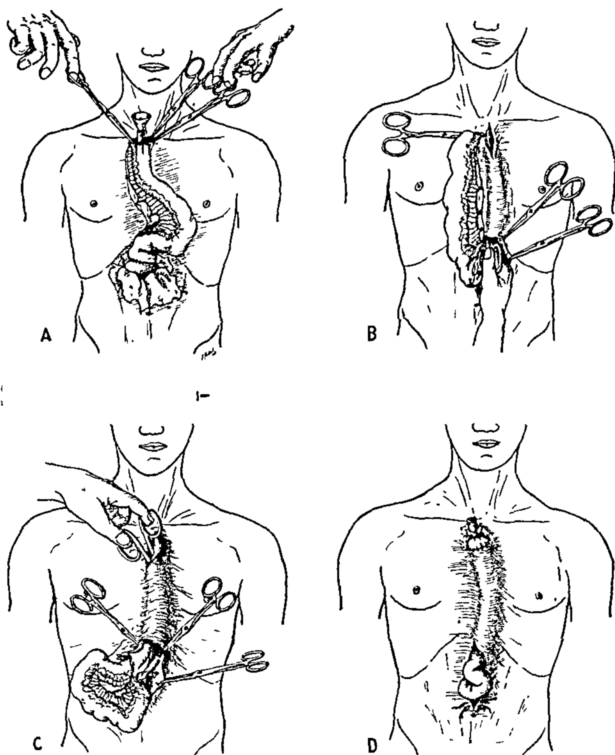


Fig. 170 Roux's method of constructing an antethoracic oesophagus with an isolated jejunal segment. A The segment has been drawn up in front of the chest and the patient is being given "un repas provisoire" B A subcutaneous tunnel has been fashioned over the sternum C The jejunal segment is being drawn into the subcutaneous tunnel D A tube has been tied into the upper end of the jejunal segment and the abdominal wound is being closed (Reproduced from *La Semaine Medicale* (1907))

procedure (Figs 169, 170) was as follows After dividing the jejunum in two places he ligated and divided four or five of the arteries leading to the vascular arcades, and the mesentery in between, at the oral end of the isolated segment, without however dividing the arcades themselves After restoring continuity of the jejunum by anastomosis with a Murphy button, and assuring himself that the remaining vascular connexions provided an adequate blood supply, he brought the isolated segment up in front of the transverse colon, anastomosed the aboral end to the stomach, and brought the other end upwards via a subcutaneous tunnel to the neck

In reporting this case Roux suggested that it would also be possible to perform oesophago or pharyngo jejunostomy "avec vidange en Y du bout superieur du jejunum," but gave no details of this procedure

Roux's report was published before the jejunal segment was anastomosed to the oesophagus, and it appears that Herzen (1908), who modified Roux's technique by bringing the jejunal segment through an opening in the transverse mesocolon instead of in front of the colon, was actually the first to complete the procedure

Lexer (1908a, 1911), and his junior colleague Frangenheim (1911, 1913, 1921, 1922), instead of trying to bridge the whole gap between the cervical oesophagus and the stomach with a jejunal segment, used a combination of antethoracic skin tube above and jejunal segment below Schreiber (1911) and Zaaijer (1913a, 1929) investigated patients radiologically after this operation and found that contrast medium was able to pass rapidly through the artificial oesophagus Sampson (1932) found that this was still true even when the patient was 'lying in an inverted position," apparently as the result of increased air pressure brought about by swallowing

Ochsner and Owens in *1934* found in the literature 36 cases of oesophagoplasty by a jejunal segment alone, and *100* by a combination of jejunal segment and skin tube Gangrene of the jejunal segment occurred in both groups, although, as might be expected, it was more common in the former,* fistula formation and stenosis, on the other hand, were *much more common in the* latter. Peptic ulceration occurred in the jejunal segment in one case reported by Hubler (1928)

Davis and Stafford (1942) reported a single case of a Lexer type oesophagoplasty in which they modified the technique by using flaps from a distance instead of local skin flaps to close the defect resulting from the construction of the antethoracic skin tube (*see also* p 261)

Yudin (1944) reported no less than 82 personal cases of antethoracic oesophagoplasty in which a jejunal segment was used Instead of isolating a segment of jejunum and attaching the lower end to the stomach, however, Yudin used a Y loop,† that is he divided the jejunum in one place only and restored continuity by end to side anastomosis as shown in Figure 171 In 21 of these cases the jejunum reached to the neck and was anastomosed directly to the oesophagus or pharynx, in 61 it failed to do so and a skin tube was required in addition

Ladd and Swenson (Ladd and Swenson, 1947, Swenson, 1947b) used a Lexer type of oesophagoplasty in children with congenital atresia of the oesophagus in whom resection and end to end anastomosis had not been *possible at the original operation* Instead of trying to bury the skin tube with local flaps they used a tubed pedicle graft from the right axilla

Longmire and Ravitch (1946) described a novel technique, which is of sufficient biological interest to merit detailed descrip

*It occurred in 8 out of the 36 cases in which a jejunal segment alone was used and in 9 out of 100 cases in which a skin tube was used in addition

†As we have seen the possibility of using this technique was foreseen by Roux

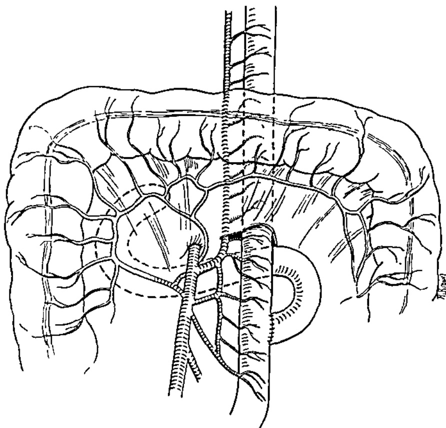


Fig 171. Modern version of the Roux loop.

tion though it has not earned a permanent place as a means of oesophagoplasty.

They began with experiments in dogs. After a segment of jejunum with a central vascular pedicle had been isolated and continuity of the bowel restored by end-to-end anastomosis, the abdomen was closed and a skin flap was outlined on the abdominal wall adjacent to the incision This flap was left attached at each end and at its centre, but was elsewhere undermined The jejunal segment was then brought out to a subcutaneous position through a stab wound located at the centre of the skin flap, and the flap was sutured to form a tube around the jejunum. The ends of the jejunal loop were brought out through stab wounds at each end of the skin tube. After six weeks the central pedicle was clamped at intervals for progressively longer periods, and when the collateral circulation seemed adequate the pedicle was divided and the mesenteric vessels were ligated. Four months later the jejunal grafts showed moderate atrophy of the musculature but were otherwise grossly normal. The mucosa continued to secrete small quantities of mucus. Peristaltic movements occurred spontaneously and also in response to mechanical stimulation.

Encouraged by these results Longmire and Ravitch constructed similar tubes of skin and jejunum in three patients. After dividing the central pedicle and ligating the mesenteric vessels they waited for a month to allow the circulation to become re-adjusted. They then began occluding the lower pedicle of the intestinal skin tube, and when the circulation through the upper pedicle appeared sufficient to support the entire tube they divided the lower pedicle and transferred it to the epigastrium. A month or so later they divided the upper pedicle, opened the skin tube along its entire length and inset it in the anterior chest

wall with the upper jejunal opening just above the left sterno-clavicular joint. Finally they anastomosed the intestinal tube to the cervical oesophagus above and to a Beck-Jianu gastric tube below. The result in the first patient—an adult with a lye stricture of the oesophagus—was encouraging. The second patient, an infant of thirteen months in whom gastric and oesophageal fistulae had been established soon after birth on account of congenital oesophageal atresia, died, and in the third patient the procedure had not been completed when their paper was published.

The idea behind this work was a sound one, and the manner in which it was applied must be reckoned as a surgical *tour de force*. The procedure has the great disadvantage, however, that it involves a series of operations spread over a long period of time, and demands surgical skill and judgement of a very high order.

Another procedure, which would seem to make even higher demands on the surgeon, was described by Longmire a year later (Longmire, 1947). In order to enable an antethoracic jejunal loop to be brought to a higher level in the neck he anastomosed a

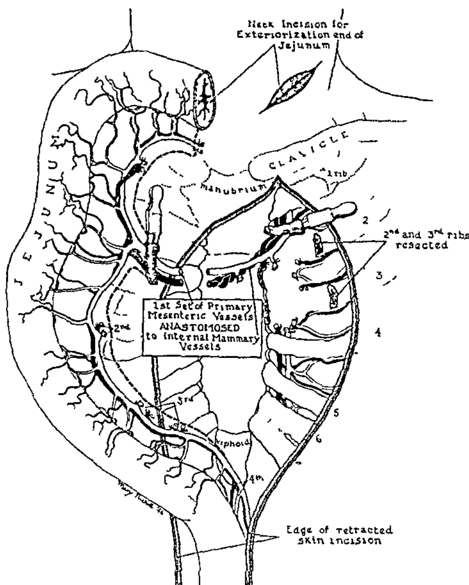


Fig 172 Longmire's modification of the Roux loop technique (Reproduced from *Surgery* by courtesy of the publishers and Dr W P Longmire)

primary mesenteric artery and the associated vein to the internal mammary artery and vein respectively (Fig. 172). The internal mammary vessels were exposed by resecting the second and third costal cartilages, and end-to-end anastomosis was performed with fine silk with the aid of a binocular loupe.

Intrathoracic Transplantation

Uffreduzzi and Giordano (1913), in experiments in dogs, succeeded in bringing up a segment of jejunum within the mediastinum to replace the oesophagus. Their achievement is the more remarkable because J. Jianu (1932) and his colleagues despite many attempts never succeeded in doing this.

Many years later Rienhoff (1944) reported what appears to be the first instance* in which a jejunal loop was brought up in the thorax in man. Rienhoff used a Y loop to short-circuit a lye stricture of the oesophagus. He made a tunnel behind the sternum by working from the abdomen below and the neck above without opening the pleura, passed the loop upwards in this tunnel, and brought the end of the loop to the surface in the second intercostal space. Subsequently he joined the jejunum to the oesophagus, first by means of a skin tube and later by direct anastomosis. The same technique was used later by Robertson and Sarjeant (1950). Harrison (1949) used a somewhat different procedure. He isolated a segment of jejunum and anastomosed the distal end to the stomach. Later through a left thoracotomy he brought the segment up via the posterior mediastinum to the neck, and at a third operation he anastomosed the jejunum to the cervical oesophagus.

Successful anastomosis of a Y-loop of jejunum to the oesophagus within the chest was first reported in 1946 by Allison in England and Rienhoff in America; prior to this, however, Sweet (1945) had reported anastomosing an inverted U-loop of jejunum to the oesophagus in the chest after total gastrectomy. Allison used the procedure in two patients to short-circuit an inoperable carcinoma of the cardia, while Rienhoff used it to restore continuity after resection of the oesophagus for carcinoma. It has subsequently been used for both these purposes and also to short-circuit benign strictures of the oesophagus (Reynolds and Young, 1948; Allison and Borrie, 1949; Allison and Da Silva, 1953). A very large series of cases was reported by Allison and Da Silva (1953), which included 28 patients with benign lesions, 44 patients with carcinoma of the oesophagus or cardia treated by radical resection, and 17 patients with inoperable carcinoma of the oesophagus or cardia. In the patients with benign lesions there were no complications referable to the loop and with few exceptions the results were excellent. In the cancer patients the results were naturally less uniform, and there were eight deaths which could be attributed to the use of a jejunal loop, three being due to gangrene of the loop and five to faulty anastomosis.

Hammer, Seay, Hill, Prust and Campbell (1955) used pedicled jejunal grafts experimentally for various purposes including reconstruction of the thoracic oesophagus. This technique has been used clinically by Brain (1953) and more recently by Allison, Wooler and Gunning (1957) to restore continuity after resection of a stricture in the lower part of the oesophagus, and by Katsura, Ishikawa and Okayama (1958) to restore continuity after resection of the middle third of the oesophagus.

Androsov (1956b) has carried a stage further the procedure devised by Longmire (1947) which we have already considered (p. 563). He excises a segment of jejunum completely, severing all its vascular connexions, and then anastomoses the main jejunal vessels to the internal mammary vessels by means of his special machine (An-

*This operation was performed in 1942.

diosov, 1956a), which unites the vessels with tantalum staples (p 421) The revascularized segment is used to create a new retrosternal oesophagus.

Intra-abdominal Oesophagojejunostomy

Transabdominal total gastrectomy for carcinoma, with re establishment of continuity by oesophagojejunostomy, was first successfully accomplished by Schlatter (1897) In the following year two cases were reported in which continuity was re-established after total gastrectomy by oesophagoduodenostomy Both operations continued to be used, though not very frequently, and by 1929 Finney and Rienhoff were able to find in the literature 26 cases of total gastrectomy and oesophagojejunostomy, of whom 14 survived the operation, and 30 cases of total gastrectomy and oesophagoduodenostomy, of whom 15 survived the operation In some of the early patients the anastomosis was made with Murphy s button, but for the most part oesophagojejunostomy was performed by making a sutured anastomosis between the end of the oesophagus and the side of a jejunal loop, and oesophagoduodenostomy by end to end sutured anastomosis after thorough mobilization of the duodenum Von Herczel (1902) anchored the jejunal loop to the diaphragm to avoid tension on the anastomosis Moynihan (1907) left the stomach attached to the oesophagus until the posterior sutures were in place so that it could be used as a tractor, and many surgeons have subsequently followed his example Schloffer (1917), Kelling (1923), Schuppel (1924) and others performed entero-anastomosis between the two limbs of the jejunal loop

Roeder (1933) collected 85 cases of total gastrectomy from the literature and added 3 of his own The operative mortality for the whole 88 cases was just 50 per cent, and the longest survival after operation was just under five years Death occurring after the immediate post operative period was most often due to recurrence of the tumour but some patients, for example Moynihan's (1907, 1911), were shown at autopsy to be free of tumour and died of nutritional anaemia Roeder indicated a strong preference for oesophagojejunostomy to re establish continuity, and emphasized the importance of anchoring the jejunal loop to the diaphragm, in addition he anchored the transverse colon to the abdominal wall if it appeared to drag on the jejunum

Cases continued to be reported (*see* e g Eusterman and Balfour, 1935, Walters, Gray and Priestley, 1942, Lahey and Marshall 1944, 1950), and in nearly all of them oesophagojejunostomy was performed, either by the technique illustrated in Figure 173 or by the modification (Fig 174) introduced by Graham (1940)

Use in Present Day Surgery

Antethoracic Transplantation

Antethoracic transplantation of jejunum, though still occasionally performed, should be regarded as obsolete

Intrathoracic Transplantation

Intrathoracic transplantation of jejunum is used for three main purposes

1 To restore continuity of the alimentary tract after radical operations for carcinoma of the cardia and the upper part of the stomach (*see* Allison and Borrie, 1949)

2 To short circuit inoperable tumours of the cardia

3. As an alternative to oesophagogastrostomy in patients with benign strictures of the oesophagus which are unsuitable for, or have not responded to, dilatation

Tumours of the cardia and upper part of the stomach are most easily approached by a left thoraco abdominal incision The Y-loop is best fashioned by the technique described in detail by Allison and Da Silva (1953) The vessels in the mesentery are lo-

cated by transillumination and the peritoneum on the aspect of the mesentery facing *the surgeon is divided from the point chosen* for division of the gut—usually four inches distal to the duodenojejunal junction—towards the root of the mesentery. The first artery supplying the arcade, and the vein or veins associated with it, are individually *ligated and divided.* The incision in the leaf of mesentery is then carried parallel to, and about 5 mm. nearer the root of the mesentery than, the arcade to the next sup-

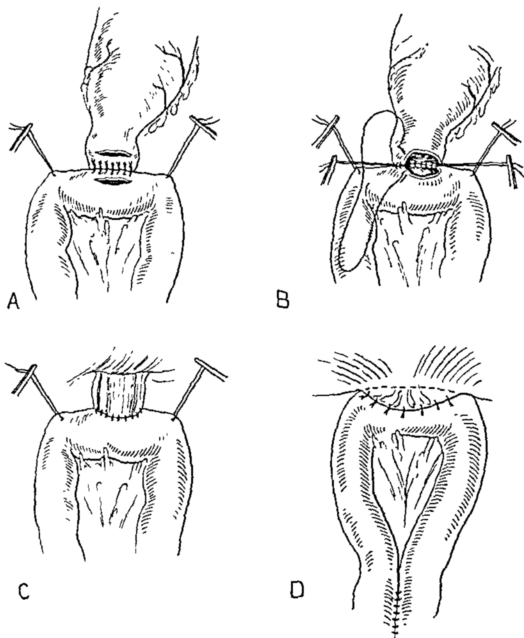


Fig 173. Oesophagojejunostomy after total gastrectomy. A. The stomach is held upwards and a row of interrupted sutures is inserted posteriorly between the muscle of the oesophagus and the serous and muscular coats of the jejunum. The oesophagus and jejunum have been opened. B. A continuous all-coats suture is inserted posteriorly and continued anteriorly as the oesophagus is gradually divided. C. The all-coats suture is reinforced by interrupted sutures uniting the muscle of the oesophagus and the serous and muscular coats of the jejunum D. A flap of peritoneum is anchored to the anterior aspect of the *jejunal loop and an entero-anastomosis is made between the limbs of the jejunal loop.*

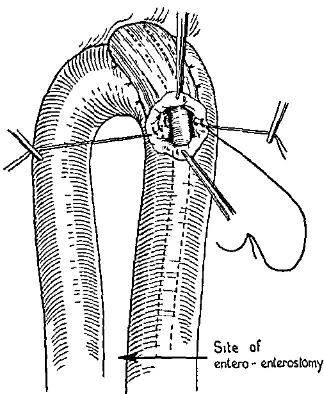


Fig 174 Graham's method of performing oesophagojejunostomy after total gastrectomy The oesophagus is united by interrupted sutures to the anterior aspect of the distal limb of the jejunal loop over a distance of about an inch The oesophagus and jejunum are then opened and the anastomosis is completed by a continuous all coats suture and interrupted sutures uniting the muscle of the oesophagus and the serous and muscular coats of the jejunum A tube is passed down the oesophagus and into the jejunum before the anastomosis is completed An entero anastomosis is made between the limbs of the jejunal loop

plying artery Fat and lymph nodes are gently stripped back, small vessels supplying the nodes being ligated or coagulated, and the second artery to the arcade and its associated veins are ligated and divided The process is continued to the next supplying trunk, and so on, until a sufficient length of viable gut has been mobilized or the circulation to the end of the segment is inadequate If the segment is not quite long enough the situation may be improved by dividing one more trunk because the gain in length due to the additional mobilization is likely to exceed the loss due to ischaemia at the end of the segment Another manoeuvre which may help is to carry the incision in the mesentery rather nearer the gut than usual, and to clear the arcades near the points of entry of the supplying vessels and allow them to hang down from the free edge of the mesentery uncovered by peritoneum

When enough vessels have been divided the peritoneum of the remaining leaf of mesentery is divided, *and the gut is divided* between fine crushing clamps at the chosen level The end of the mobilized segment is closed without excessive inversion, and continuity of the jejunum is restored by end to side anastomosis (*see* Fig 171) After radical gastrectomy the segment is passed through an opening in the transverse mesocolon and through the enlarged oesophageal hiatus to the posterior mediastinum In palliative oesophagojejunostomy for irremovable carcinoma of the cardia the oesophagus is divided above the lesion and the distal end is closed The mobilized jejunal segment is passed through a new opening made in the diaphragm just above the external arcuate ligament to the left pleural cavity, and then to the mediastinum The openings in the mesocolon and diaphragm are closed snugly about the jejunal segment and its mesentery, and the proximal end of the oesophagus is anastomosed to the anterior aspect of the jejunal segment

In patients with benign stricture of the oesophagus, jejuno oesophagoplasty with a Y loop has the disadvantage that it excludes a healthy stomach from the digestive tract, and for this reason many surgeons prefer oesophagogastrostomy when operation is required in these patients Another procedure which, as we have seen, has been used by Brain (1953) and Allison, Wooler and Gunning (1957), and which they consider particularly suitable for use in children after resecting a benign stricture, is to restore continuity with a pedicled segment of jejunum.

Intra-abdominal Oesophagojejunostomy

Transabdominal total gastrectomy, though strongly advocated by Lahey and his colleagues (Lahey, 1938, 1950; Lahey and Marshall, 1944, 1950) as the treatment of choice in early cancer of the stomach, is an uncommon operation in most clinics. The operative mortality has been reduced and the task of maintaining nutrition after operation is now much easier than it was, but most surgeons prefer to conserve some stomach whenever possible Moreover when total gastrectomy is indicated a thoracic or thoraco-abdominal approach is usually employed

When transabdominal total gastrectomy is performed continuity of the digestive tract is now nearly always re-established by oesophagojejunostomy A loop of jejunum is brought up, usually in front of the transverse colon, anchored to the diaphragm, and anastomosed to the oesophagus by one of the two techniques illustrated in Figures 173 and 174 Entero-anastomosis is usually performed between the afferent and efferent limbs of the jejunal loop, and is essential when Graham's technique is used for the oesophagojejunal anastomosis

TRANSPLANTATION OF COLON

History

Antethoracic Transplantation

Kelling in 1911 reported a case in which he transplanted the transverse colon to short-circuit a carcinoma of the oesophagus. As the first stage he divided the colon proximally and distally, and the great omentum, while preserving the transverse mesocolon and the middle colic vessels. After restoring continuity of the colon he anastomosed the distal end of the isolated segment to the stomach and carried the proximal end upwards in a subcutaneous tunnel to the level of the nipple. Three weeks later he established a cervical oesophagostomy and prolonged the colon transplant with a skin tube. He planned to join the cervical oesophagus to the skin tube but the patient died before this was done.

In the same year Vulliet (1911) suggested transplanting the transverse colon with an antiperistaltic orientation, and this procedure was subsequently used successfully by von Hacker (1914b) in a child with a lye stricture of the oesophagus.

Antethoracic isoperistaltic transplantation of the right half of the colon was initiated by Roith (1923), and according to Godard (1951) was used subsequently in one case by Leveuf between 1933 and 1936.

These operations for many years attracted little attention, but interest in the subject was revived by the work of Orsoni and other French surgeons, who used the descending colon and the left half of the transverse colon to construct an antethoracic short-circuit between the cervical oesophagus and the stomach (Orsoni and Toupet, 1950; Orsoni, 1951; Orsoni and Lemaire, 1951; Rudler, 1951a, Lafargue, Dufour, Cabanie and Chavannaz, 1951a, b) or jejunum (Poilleux and Frileux, 1950) in patients with inoperable carcinoma of the oesophagus or cardia, and occasionally as a preliminary procedure in poor-risk patients with operable tumours. The mortality was high, and there was moreover a high incidence of fistulae in the survivors (Rudler, 1951b, c), but some of the results were sufficiently encouraging to stimulate further work.

Orsoni himself suggested in 1951 that anastomosis between the colon and pharynx was possible, and in 1954 Goligher and Robin proved this by successfully using the left colon to connect the pharyngeal remnant to the stomach after pharyngolaryngectomy for carcinoma of the hypopharynx in a man of 34 The operation was performed in two stages. At the first stage the left colic, accessory left colic and first sigmoid vessels were divided. Five weeks later* one surgeon

*It had been intended to allow two weeks between

→

performed pharyngolaryngectomy and bilateral block dissection of the cervical lymph nodes, while another reopened the abdomen and completed the mobilization of the left colon The colon and marginal vessels were divided at the right end of the transverse colon and at the middle of the sigmoid loop It had been hoped to restore continuity by mobilizing the right colon sufficiently for it to be anastomosed to the sigmoid but this was not feasible, the right colon was therefore excised and the ileum was anastomosed to the sigmoid colon The sigmoid end of the isolated loop of colon was drawn upwards behind the stomach, through the lesser omentum, through the incision in the abdominal wall, and then in a subcutaneous tunnel to the neck just to the left of the midline When fully extended the segment was found to stretch almost to the top of the head The excess was amputated and the colon was then anastomosed end to end to the pharyngeal remnant The left sternomastoid muscle was divided to provide more room for the colon as it crossed from the front of the chest to the neck The cut end of the trachea was sutured to the skin in the suprasternal notch and the cervical wound was then closed Finally the other end of the colon transplant was anastomosed to the stomach (Fig 175)

The patient made a good recovery but developed a recurrence two and a half months later In the interim swallowing was satisfactory but, as might be expected, progress with speech was very slow

Intrathoracic Transplantation

Orsoni (1951) stated that he regarded his work on antethoracic transplantation of the colon as merely a preliminary to intrathoracic transplantation He suggested that intrathoracic colon transplants might be used in three ways to connect the cervical oesophagus to the jejunum, to connect the upper part of the thoracic oesophagus to the lower part, and to connect the cervical oesophagus to the lower thoracic oesophagus For congenital oesophageal atresia he proposed using the second technique, for benign strictures he proposed the first or third technique, and for restoration of continuity after resection of the thoracic oesophagus for carcinoma he proposed the first technique He suggested further that antethoracic transplantation of the colon might continue to be used to short-circuit inoperable tumours of the upper thoracic oesophagus, and for reconstruction after resection of tumours of the cervical oesophagus

In the same year Rudler and Monod Broca (1951) transplanted the right colon and terminal ileum, and Lortat Jacob (1951) transplanted the ascending and transverse colon, to short circuit a benign stricture In both these cases the transplant was orientated isoperistaltically and was anastomosed below to the jejunum Rudler and Monod Broca brought the transplant up retrosternally to the neck, whereas in Lortat Jacob's case the transplant was anastomosed above to the oesophagus in the posterior mediastinum

Kergin (1953) transplanted the transverse colon isoperistaltically and retrosternally to bypass a stricture of the oesophagus due to paraffinoma anastomosing the transplant to the cervical oesophagus above and to the thoracic oesophagus below

Mahoney and Sherman (1954) used the right colon to restore continuity after resecting the thoracic oesophagus for carcinoma The operation was performed in one session though it involved four steps First of all the right pleural cavity was opened and the whole oesophagus was mobilized after which the chest was temporarily closed Next the abdomen was opened through an upper midline incision and the right half of the colon was mobilized The ileocolic artery was ligated and divided at its origin The terminal ileum was divided and the distal end was

stages but the patient's wife was pregnant and the second stage was postponed until after her confinement

inverted, after which the transverse colon was divided to the left of the midline and the continuity of the bowel was restored by end-to-end ileocolostomy. The distal end of the isolated segment of colon was then anastomosed to the stomach Thirdly, the chest was re-opened and the oesophagus was divided at the cardia. The distal end of the oesophagus was inverted and the proxi end was brought out through an incision the neck. Finally, a retrosternal tunnel prepared by blunt and sharp dissection, a the caecal end of the transplant was brou up and anastomosed to the divided oesop gus in the neck. After a stormy post-ope tive course the patient made good progr

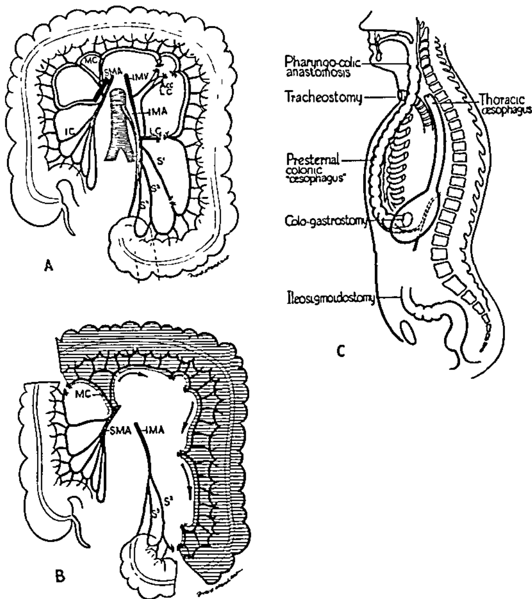


Fig. 175 Reconstruction of the alimentary tract after pharyngolaryngectomy by transplantation of the colon. A First stage of preparation of the colon Ligation and division of left colic (L C), accessory left colic (Acc L C) and first sigmoid (S^1) vessels. B. Second stage of preparation of the colon. Division of marginal vessels and colon at right end of transverse colon and middle of sigmoid loop, leaving left colon entirely dependent on the middle colic (M C) artery for its blood supply. C Diagram showing the pre sternal situation of the left colon at the conclusion of the procedure. (Reproduced from the *British Journal of Surgery* by courtesy of the publishers and Professor J. C. Goligher.)

but died four months after operation apparently because of recurrence of the tumour

Dale and Sherman (1955) transplanted the right colon retrosternally to restore continuity of the alimentary tract in two young children who had been operated on soon after birth for congenital oesophageal atresia, and left with oesophageal and gastric fistulae because end to end anastomosis of the oesophagus was not feasible The cervical anastomosis had to be revised in one of the patients but apart from this recovery was uneventful In both the colon was prepared for operation by administering Neomycin and Sulphathalidine The caecal end of the mobilized right colon was passed upward in a retrosternal tunnel made by working from above and below without opening the pleura, and was anastomosed to the oesophagus in the neck The lower end of the colon transplant was anastomosed to the stomach, the gastrostomy being retained until oral feeding was established

Sherman and Waterston (1957) used the transverse colon to bridge the gap between the cervical oesophagus and the lower oesophagus as a secondary procedure in children with congenital oesophageal atresia The transverse colon has also been used in five children to restore continuity after resection of the distal part of the oesophagus and the proximal part of the stomach for portal hypertension due to extrahepatic portal obstruction (Koop and Roddy, 1958), but it remains to be seen whether further varices will develop in the submucosa of the colon as new collateral pathways are formed Finally, Montenegro and Cutait (1958) have used the transverse colon to restore continuity in 26 cases following either partial or total oesophagectomy for benign stricture, oesophageal varices or carcinoma, the colon being brought up into the cervical region through the pleural cavity (Figs 176, 177)

Use in Present Day Surgery

Transplantation of the colon to replace the whole or part of the oesophagus must be regarded as still *sub judice* The colon, unlike the small bowel, possesses a marginal artery which is almost as long as the bowel itself, and can unquestionably provide a longer transplant than the jejunum Since modern methods of reducing the intestinal flora have removed the main hazard of operating on the colon it seems likely that colon transplants will be used increasingly to restore continuity of the alimentary tract when the upper junction has to be made to the cervical oesophagus or the pharynx

TRANSPLANTATION OF JEJUNUM AND COLON TO THE STOMACH

We shall consider four types of procedure

1 Gastrojejunostomy without resection of the stomach

2 Gastrojejunostomy after resection of the stomach

3 Partial replacement of the stomach by jejunum

4 Partial replacement of the stomach by colon

GASTROJEJUNOSTOMY WITHOUT RESECTION OF THE STOMACH

History

Gastrojejunostomy was first performed by Wolfler in 1881 Wolfler, who was working in Billroth's clinic, was operating on a patient with a carcinoma of the pylorus and had found that the tumour was irremovable He was about to close the abdomen when his

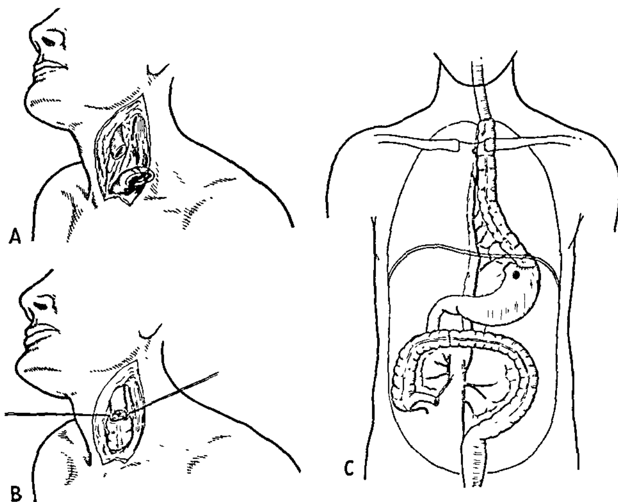


Fig 176 Technique of Montenegro and Cutait for constructing a substitute oesophagus from the transverse colon A, B Anastomosis between the cervical oesophagus and the colon. C The colon has been anastomosed to the cervical oesophagus above and to the stomach below. (Reproduced from *Surgery* by courtesy of the publishers and Dr E B. Montenegro)

assistant, Nicoladoni, suggested that the stomach could be provided with a new outlet by anastomosing it to the small bowel Wölfler did this by bringing up a long loop anterior to the colon and anastomosing it antiperistaltically* to the anterior aspect of the stomach.

A few years later von Hacker (1885) introduced a new technique, posterior gastrojejunostomy, in which he brought a long loop of jejunum up through the transverse mesocolon and anastomosed it antiperistaltically to the posterior aspect of the stomach

*i e with the proximal end of the jejunal loop nearest to the pylorus

Another modification, isoperistaltic anastomosis, was introduced by Rockwitz (1887), and subsequently Brenner (1892) brought up a long loop through the mesocolon and the gastrocolic omentum and anastomosed it isoperistaltically to the anterior aspect of the stomach

It was thought, when gastrojejunostomy was introduced, that carcinoma of the stomach was the main cause of pyloric obstruction. When it was realized that pyloric obstruction was often due to duodenal ulcer, gastrojejunostomy began to be used in these cases also. Finney reported one such operation in 1893, but his account rather suggests

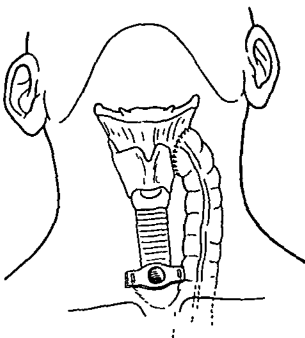


Fig 177 Technique of Montenegro and Cutait for replacement of the whole oesophagus by transverse colon Anastomosis between the hypopharynx and the colon (Reproduced from *Surgery* by courtesy of the publishers and Dr L B Montenegro)

that this was not the first occasion on which gastrojejunostomy had been performed for non malignant pyloric obstruction The next step, which appears to have been taken first by Doyen (1894, 1898), was to try the operation in ulcer patients in whom there was no obstruction

Gastrojejunostomy by the techniques so far described, whether performed for carcinoma or peptic ulcer, was often followed by severe and persistent vomiting * To prevent this Braun (1893) anastomosed the afferent and efferent limbs of the jejunal loop Jaboulay (1892a, b) anastomosed the jejunum below the stoma to the third part of the duodenum Doyen (1898), in addition to performing entero anastomosis, divided and closed the afferent limb between the gastric stoma and the entero anastomosis Roux (1897) performed gastrojejunostomy *en Y* (Fig 178) He did not claim originality for the operation but attributed it to Socin, who in turn attributed it to Wolfler Lucke (1899) modified Roux's technique by closing the end of the jejunum and making the anastomosis to the stomach side to side

These operations avoided one complication but resulted in a high incidence of another, namely, jejunal ulceration Some other means of avoiding vicious circle vomiting was therefore required and this was provided by the so called posterior no loop operation introduced by Petersen of Czerny's clinic in 1901 It is of course impossible to perform a strictly no loop gastrojejunostomy, but Petersen made the loop as short as possible His operation was adopted enthusiastically, notably by the Mayos in America and by Moynihan in England W. J Mayo (1906) preferred antiperistaltic anastomosis with an oblique stoma, and Moynihan (1908) a vertical stoma, but it was generally agreed that provided the loop was short the direction of the stoma and the orientation of the jejunum did not greatly matter

It was soon found that gastrojejunostomy did not constitute a panacea for peptic ulcer in general, and that the best results were obtained in patients with pyloric obstruction (*see* Moynihan, 1908) In consequence partial gastrectomy began to be used to an increasing extent for ulcers not complicated by obstruction but which failed nevertheless to respond to medical treatment

Use in Present Day Surgery

Gastrojejunostomy is the operation of choice in elderly poor risk patients* with pyloric obstruction due to scarring associated with a duodenal ulcer These patients, in contrast to younger patients with active duodenal ulceration, usually have quite a low concentration of free hydrochloric acid in the stomach and the risk of further ulceration at the stoma or in the jejunal loop is small

In gastric and duodenal ulcer not asso

*Often referred to as vicious circle vomiting

* After proper preparation

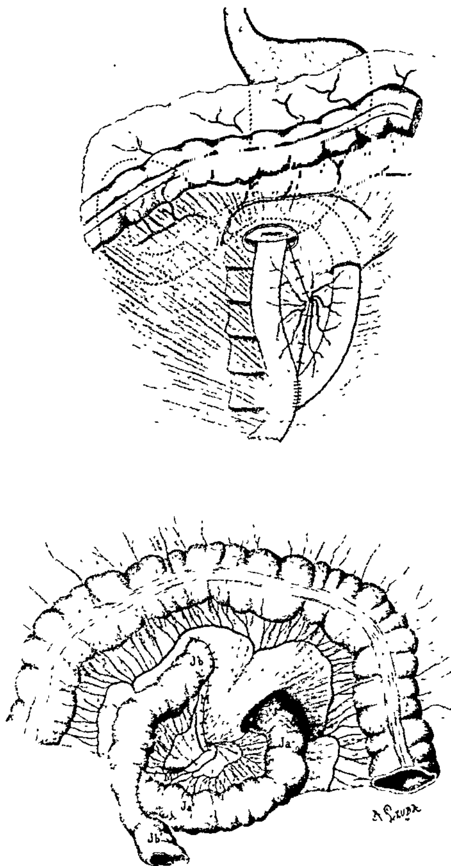


Fig 178 Gastrojejunostomy en Y. (Two of Roux's original illustrations reproduced from *Revue de Gynécologie et de Chirurgie Abdominale*, Vol. 1, p 67, 1897, by courtesy of the publishers)

ciated with organic obstruction there is a considerable risk of stomal or jejunal ulcer developing after gastrojejunostomy alone. If operation is required partial gastrectomy is usually performed but some surgeons combine gastrojejunostomy with vagotomy for the treatment of duodenal ulcer.

Gastrojejunostomy is occasionally indicated as a palliative procedure in carcinoma of the stomach but a resection should be performed whenever possible even when distant metastasis has occurred.

In ulcer patients the operation is usually of the posterior short loop variety. In patients with cancer anterior gastrojejunostomy is usually preferred because obstruction of the loop as the result of spread of the tumour is likely to occur sooner if the posterior operation is performed.

GASTROJEJUNAL ANASTOMOSIS AFTER PARTIAL GASTRECTOMY

History

The first successful partial gastrectomy was performed by Billroth in 1881, but he re-established continuity by end to end anastomosis of the stomach to the duodenum. This procedure is still known as the Billroth I operation though it had been performed unsuccessfully by Péan (1879) and Rydygier (1880) before it was used by Billroth. Re-establishment of continuity after partial gastrectomy by gastrojejunostomy—known still as the Billroth II operation—was initiated by Billroth and performed successfully by him in 1885. After resecting the pyloric part of the stomach which was the site of a carcinoma he closed the cut ends of the stomach and duodenum and then brought up a long loop of jejunum in front of the colon and anastomosed it to the most dependent part of the stomach.

The notion of utilizing the cut end of the stomach for the anastomosis to the jejunum is often attributed to Polya but was first suggested by von Hacker (1885) and this procedure, which is usually known as the Polya operation was performed by various surgeons including Kronlein (1888), von Eiselsberg (1889) and Reichel (1908 *see also* 1911) before Polya's paper was published in 1911. There seems to be no doubt that the surgeons concerned were unaware of each other's work principally because as Polya (1940) himself has recently pointed out their cases were presented usually in casuistical publications and in demonstrations before medical societies which generally do not awake public interest.

Many variants of the Polya operation have been devised. The jejunal loop has been brought up anterior to the transverse colon and posteriorly through an opening in the mesocolon and the anastomosis has been made with part of the width of the stomach as well as with the whole width. The operation in which only part of the width of the stomach is used for the anastomosis is often attributed to Hofmeister but appears to have been performed first by von Eiselsberg (1889) and the use of a long loop of jejunum anterior to the colon with entero-anastomosis between the afferent and efferent limbs is commonly attributed to Balfour (1917) though it appears to have been introduced by Hofmeister (*see* Walters Gray and Priestley 1942 p 364); it is however extremely difficult and not of great importance to assign priorities for the various modifications.

Use in Present Day Surgery

The Billroth II operation in its original form is now obsolete but the Polya operation and its modifications are widely used in the treatment of carcinoma of the stomach and peptic ulcer.* Even in patients with duodenal ulcer the risk of post-operative stomal and jejunal ulceration is small pro

*It would be out of place to discuss at length the relative merits of the Billroth I and Polya operations. The author's preference is for the Billroth I in gastric ulcer but for the Polya operation in duodenal ulcer.

vided that about two-thirds of the stomach including the whole of the pyloric antrum —or at least the whole of the mucosa of the pyloric antrum—is removed, because the number of acid-secreting cells is considerably reduced and the chemical (though not the nervous) stimulus to acid secretion is abolished.

PARTIAL REPLACEMENT OF THE STOMACH BY JEJUNUM

History

Fiori (1913) transected the pyloric antrum in three dogs and re-established continuity with a pedicled segment of jejunum. The dogs survived for 5, 22 and 43 days respectively. The cause of death was not stated but the transplants appeared healthy and showed no ulceration.

Mann (1916) inserted small pedicle transplants, consisting of the full thickness of the wall of the jejunum with the mucosal surface inwards, as patches in defects made in the wall of the stomach in dogs. He found that with one exception the transplants remained healthy and free from ulceration even when necrosis and ulceration occurred in the stomach itself in response to subsequent bilateral adrenalectomy. Some years later Dragstedt and Vaughan (1924) confirmed that pedicle transplants of jejunal wall in dogs resisted digestion when inserted in defects in the wall of the stomach with the mucosal surface inwards, but found that digestion occurred rapidly if the orientation was reversed so that the serosal surface of the patch was on the inside.† Subsequently ulceration was observed in a proportion of jejunal transplants exposed to gastric juice by De Takats and Mann (1927) and Morton (1927), and more recently Price and Lee (1946) reported *inter alia* that the mucosa of jejunal pedicle transplants was not completely resistant to gastric digestion though it was much more resistant than the serosal surface of the gut. Saltzstein and Kurtz (1946), on the other hand, found that the mucosa of jejunal pedicle transplants to the stomachs of Mann-Williamson dogs‡ remained intact even though the jejunum distal to the gastrojejunostomy consistently ulcerated.

Andrus and his colleagues (Stefko, Andrus and Lord, 1942; Andrus, Lord and Stefko, 1943a) measured the pH of aspirated gastric contents and of the mucosa in various parts of the stomach in dogs before and after (a) gastrojejunostomy and (b) insertion of a jejunal pedicle graft to a defect in the stomach wall, and also the change in pH resulting from injection of histamine. They found that gastrojejunostomy alone had little or no effect on gastric acidity, nor did it prevent a marked increase in acidity in response to histamine. A jejunal pedicle graft, on the other hand, resulted in decreased gastric acidity, which was demonstrable within forty-five minutes of transplantation, persisted for at least four months, and ceased within an hour if the graft was removed. In addition, administration of histamine to animals with jejunal pedicle transplants caused a fall instead of a rise in gastric acidity. Pedicle grafts of duodenum had a similar effect, but pedicle grafts of ileum and colon did not.

In the light of their experimental findings Andrus *et al.* (1943b) made jejunal pedicle transplants to the wall of the stomach in four patients with peptic ulcer. In three of the patients the acidity of the gastric mucosa was reduced, and in the fourth patient there

†Previously Katzenstein (1908) and Kathe (1908) had transplanted pedicled loops of jejunum into the lumen of the stomach in dogs and had found that digestion occurred rapidly. This was subsequently denied by Hotz (1910) and Licini (1912), but was confirmed by Best (1914).

‡i.e. dogs subjected to the procedure designed by Mann and Williamson (1923) for the experimental production of peptic ulcer. In this procedure the pylorus is divided and the duodenal stump is closed. The jejunum is then transected and the proximal end is anastomosed to the ileum. The distal end of the jejunum is anastomosed end to end to the stomach.

was clinical improvement but no reduction in acidity. A long-term follow up of eleven other cases* treated in the same way, however, showed that the gastric acidity had returned to normal and several patients had developed ulcers in the graft (Moore, 1956).

Henley (1952, 1953) used a pedicled segment of jejunum to replace the portion of stomach removed in partial gastrectomy, as a primary procedure and also as a secondary procedure in patients in whom a diagnosis of post-gastrectomy syndrome had been made following a Polya-type gastrectomy. In 1953 he reported a total of 73 ulcer patients treated in this way, 61 of whom had been followed sufficiently long for the value of the procedure to be assessed. Three of the patients developed another ulcer either in the stomach itself or in the transplant, but generally speaking the results were regarded as excellent.

Henley has also used a jejunal segment to connect the oesophagus to the duodenum after total gastrectomy.

Use in Present Day Surgery

Insertion of jejunal pedicle transplants in the stomach wall, as we have seen, has been shown to be ineffective and has no place in the treatment of peptic ulcer.

Henley's operation, according to its originator, offers the advantages of the Billroth I without its disadvantages, and can be performed when the Billroth I is technically impossible. It is, however, complicated and time-consuming, and even in Henley's own series was occasionally followed by recurrent ulceration. The risk of this happening can be reduced by removing a greater proportion of stomach, but unduly radical gastrectomy is likely to be followed by nutritional disturbances. For these reasons few surgeons have adopted Henley's procedure.

*As Moore (1956) pointed out, Dr. Andrus died in 1951 after a long illness and before he was able to publish further follow-up studies on the patients treated by his group.

PARTIAL REPLACEMENT OF THE STOMACH BY COLON

History

It was reported by Dragstedt and Vaughan (1924) that pedicle transplants of the wall of the colon, inserted into defects in the wall of the stomach in dogs, did not ulcerate. More recently however Sircus (1956) showed that a pedicled segment of colon transplanted to the defect after transecting, or resecting part of, the stomach in dogs was very prone to peptic ulceration. The incidence of ulcers in such transplants was increased by daily injection of histamine in beeswax, and was decreased when the area of parietal-cell-bearing mucosa proximal to the transplant was small, or when bilateral vagotomy was added to the procedure.

In man there has been some difference of opinion concerning the capacity of the colon to resist peptic digestion. Wilkie (1931) reported a case of gastrojejunocolic fistula in which peptic ulceration occurred in the colon. Eggers (1939), on the other hand, successfully treated a patient with this condition by transecting the colon proximal and distal to the fistula, closing the ends of the isolated segment without separating it from the stomach, and re-establishing continuity of the colon by end-to-end anastomosis.

Moroney (1948) reported another case similar to that of Eggers, and the fact that the segment of colon left in communication with the stomach remained free of ulceration encouraged him (Moroney, 1951, 1953) to use a segment of colon to reconstruct the stomach after partial gastrectomy. The colon, he pointed out, secretes protective mucus, is fairly capacious, plays no active part in the digestion and absorption of food, and its mucous membrane is normally slightly acid in reaction; in addition it can provide satisfactory replacement even when transplanted antiperistaltically. On the other hand the "jejunal a deprived

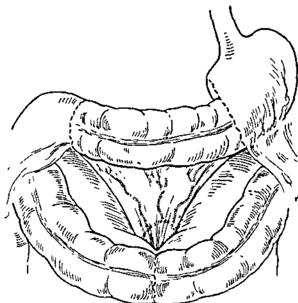


Fig 179 Moroney's method of restoring continuity after partial gastrectomy by means of a segment of transverse colon

the neutralizing and diluting action of pancreatic juice is well known to be particularly susceptible to ulceration .. To use a loop of jejunum between the gastric remnant and duodenum seemed to expose it to perils identical with, or even greater than, those which have followed entero-enterostomy" (Moroney, 1953)

Moroney has performed colonic replacement as a primary procedure in patients with gastric and duodenal ulcer and carcinoma of the stomach, and as a secondary procedure in patients with post-gastrectomy syndromes following operations of the Polya type In his Hunterian lecture (Moroney, 1953) he described his technique in detail (Fig. 179) and reported 150 cases. In this series there were 7 deaths; in the remaining 143 patients the result was regarded as satisfactory in 125 and unsatisfactory in 18. Recurrent ulcer was proved to have occurred in 3 patients and was suspected in 2 others; in all but one of these, however, only about two-thirds of the stomach was removed, and this, in Moroney's view, is insufficient when colonic replacement is undertaken in patients with duodenal ulcer.

Harrison (1952) studied 30 of Moroney's patients radiologically. He found that the colon transplant showed active movement and frequently regurgitated ingested material into the stomach remnant, thus ensuring thorough mixing. Passage of a meal through the small bowel was a little slower than normal.

Use in Present Day Surgery

Despite Moroney's advocacy colonic replacement of the stomach has not been widely used, and has indeed been roundly condemned by some surgeons of experience (e g Ogilvie, 1956). In the author's view it is not justifiable as a primary procedure but may occasionally be indicated as a secondary procedure in patients with severe and intractable post-gastrectomy symptoms. If the operation is performed in patients with high gastric acidity it is essential to reduce the acidity to a low level by generous resection of the stomach or by adding vagotomy to the operation.

ANASTOMOSIS BETWEEN THE BILIARY AND GASTRO-INTESTINAL TRACTS

Anastomosis between the biliary and gastro-intestinal tracts is used to bypass obstructions of the biliary tract due to various causes, and to re-establish continuity of the biliary tract after pancreaticoduodenectomy. It has also been tried for the treatment of peptic ulcer and gallstones, though it is never used for such purposes nowadays.

We shall consider the following procedures:

1 Anastomosis between the gall bladder and the gastro-intestinal tract.

2 Anastomosis between the common bile duct and the gastro intestinal tract

3 Anastomosis between the hepatic ducts and the gastro intestinal tract

4 Anastomosis of a biliary fistula to the gastro intestinal tract.

5 Anastomosis between intrahepatic ducts and the gastro intestinal tract

ANASTOMOSIS BETWEEN THE GALL BLADDER AND THE GASTRO-INTESTINAL TRACT

History

Von Winiwarter (1882) anastomosed the gall bladder to the colon, and this appears to be the first short circuiting operation for the relief of biliary obstruction of which there is any record It took seven operations over a period of two years to complete the procedure and deal with the various complications which occurred Witzel (1885) published a critical account of von Winiwarter's case and suggested that it might be possible to make such an anastomosis in one operation

Monastyrski, according to Redell (1940), performed the first successful cholecystjejunostomy in 1887 (*see* Monastyrski, 1888), but the first case to be reported is that of Kappeler (1887, 1889), whose patient was operated on a few weeks later Thereafter reports of cholecystjejunostomy and cholecystduodenostomy were published by many surgeons, including Weir (1893), Codivilla (1898), Halsted (1899), Merk (1902), Robson (1904), Korte (1905), Arnsperger (1906) and Quenu (1909) In some of these cases Murphy's button was used, in others the anastomosis was by suture As a rule the operation was performed to short circuit a benign stricture or as a palliative procedure in patients with carcinoma of the head of the pancreas or the ampulla of Vater, Codivilla (1898) and Halsted (1899), however, performed it as a preliminary to partial pancreaticoduodenectomy,* and their example has been followed much more recently by Brunschwig (1937) Klinkert (1929) and Lauwers (1933) performed cholecystjejunostomy after transduodenal resection of a carcinoma of the ampulla of Vater

When the operation was first introduced the mortality varied greatly and was often high even in patients with benign strictures, it was therefore a noteworthy feat when Deaver (cited Deaver and Ashhurst, 1914) performed twenty successive operations without a death

A considerable number of patients who survived the operation later developed ascending infection of the biliary tract and liver To diminish the risk of this complication Krause (cited by Maragliano, 1903), after performing cholecystjejunostomy, anastomosed the afferent and efferent limbs of the jejunal loop, more recently Peterson and Cole (1948), finding that this was not a sufficient safeguard in patients with benign lesions, and therefore as a rule quite a long expectation of life, advocated in addition creating three folds in the afferent limb Monprofit (1904, 1908) constructed a Y loop of jejunum by Roux's technique (p 561) and anastomosed this to the gall bladder, and his procedure has since been used extensively (*see* e g Lahey, 1940)

Cholecystgastrostomy appears to have been first performed clinically by Mayo Robson (1890), though it had previously been used experimentally in dogs by Oddi (1888) Robson s patient died, and the first successful cholecystgastrostomy in man appears to be that reported by Wickhoff and Angelberger (1893) Further experiments in dogs were reported by Dastre (1890),

*Codivilla s patient lived only twenty four days Halsted whose patient lived for seven months after completion of the operation appears to have been the first to perform pancreaticoduodenectomy Several later writers (e g Loggan and Kleinsasser 1951) suggest that the operation had been performed successfully by Billroth some years earlier but the author has not found *any convincing* evidence of this

Cannac (1897) and Masse (1898). Further cases were reported by Terrier (1896), Monod (1896), Jaboulay (1898), Montognon and Duchamp (1899), Hildebrand (1902, 1903), Eichmeyer (1910, 1911) and Kehr (1913a, b, 1914), and the operation came to be used extensively as a short-circuiting procedure for the relief of both malignant and non-malignant biliary obstruction (*see* Gentile, 1935, for review) The risk of subsequent cholangitis, which deterred many of the earlier surgeons, was later seen to have been exaggerated (Judd, 1928, Wangensteen, 1928, Mallet-Guy, 1933; Redell, 1940). It is however by no means negligible, and in the hope of reducing it Mason (1930), DeBakey and Ochsner (1939), and Zollinger (1940) devised methods of valvular cholecystgastrostomy.

Whipple, Parsons and Mullins (1935), whose work marks the beginning of the modern approach to the treatment of cancer of the ampulla of Vater, performed cholecystgastrostomy in the first stage of their two-stage pancreaticoduodenectomy, and their technique has since been used by various other surgeons. Hunt (1941) used cholecystgastrostomy as part of a one-stage pancreaticoduodenectomy; nowadays, however, while a one stage procedure is preferred by most surgeons, the flow of bile is re-established by anastomosis of the common bile duct to the gastro-intestinal tract (*v. infra*)

Cholecystgastrostomy was tried for the treatment of peptic ulcer by Babcock (1920), Frenkel (1925), Deaver (1925), Braithwaite (1926), Nazarov (1927) and others, and was suggested for the treatment of cholecystitis and cholangitis not due to calculi by DuBose (1924), much better methods of treating these conditions are, however, now available

Use in Present Day Surgery

Anastomosis between the gall bladder and the gastro-intestinal tract is widely used as a palliative procedure in biliary obstruction due to carcinoma of the head of the pancreas or the ampulla of Vater. Cholecystduodenostomy has the disadvantage that the anastomosis lies close to the tumour and may soon become obstructed, and many surgeons dislike cholecystgastrostomy because the stomach is so thick-walled; the usual procedure therefore is cholecystjejunostomy, using either a simple or a Y-loop of jejunum.

Cholecystjejunostomy, or occasionally cholecystgastrostomy, is performed as a first-stage procedure when pancreaticoduodenectomy is to be carried out in two stages. In the more common one-stage operation however choledochojejunostomy is preferable.

In chronic pancreatitis anastomosis between the biliary and gastro-intestinal tracts is sometimes indicated. Some form of choledocho-enterostomy (p. 581) is then the usual procedure, but occasionally the gall bladder is used for the anastomosis.

In patients with stricture of the common bile duct due to injury or inflammation the gall bladder has usually been removed If present it may be used in a short-circuiting operation but this is rarely if ever the treatment of choice (p. 582).

In congenital atresia of the bile ducts the gall bladder can be used in a short-circuiting operation if it contains bile. This, however, is not a very common finding,* and when it is present choledochoduodenostomy is also feasible, and is to be preferred.

ANASTOMOSIS BETWEEN THE COMMON BILE DUCT AND THE GASTRO-INTESTINAL TRACT

History

Anastomosis between the common bile duct and the duodenum (choledochoduodenostomy) appears to have been first performed by Riedel in 1888 for calculous obstruction, though it was not reported until some years later (Riedel, 1892) Riedel's pa-

*It occurred in 16 out of the 146 cases in Gross' (1953) series

tient survived only one day, but cases were reported subsequently by Sprengel (1891), Mayo Robson (1904), W J Mayo (1905), Fullerton (1907), Packard (1908), Sasse (1913) and others in which choledochoduodenostomy was used successfully to relieve obstruction due to stricture of the common bile duct or carcinoma of the pancreas In most of these cases *side to side anastomosis* was used, but complete division of the duct followed by end to side anastomosis, which Deaver and Ashhurst (1914) attributed to Czerny, gradually gained in popularity Walton (1915) introduced a new technique in which he anastomosed the end of the duct to a tube fashioned from a pedicled flap of duodenal wall

Halsted (1899), in a celebrated case, anastomosed the common bile duct to the duodenum after performing partial pancreaticoduodenectomy, but he also performed cholecystostomy, and later cholecystduodenostomy, in the same patient Hirschel (1914) and Tenani (1922) used choledochoduodenostomy as the definitive means of re establishing biliary flow after pancreaticoduodenectomy, but this procedure is not applicable after the more radical resection employed today

Swain (1895) anastomosed what he thought was a grossly dilated common bile duct side to side to the jejunum as a short circuiting procedure Jackson (1914), in a patient with a stricture of the common bile duct, anastomosed the end of the duct to a simple loop of jejunum brought up in front of the transverse colon,* and Kausch (1911) anastomosed the duct to a Y loop to relieve obstruction due to a lesion of the ampulla of unknown etiology Coffey (1909) devised a method of anastomosing the common bile duct to the jejunum in dogs in which the lowest inch of the duct was buried between the mucosa and the circular muscle of the gut He used a similar procedure for anastomosing the ureter to the colon (*see* Fig. 187) This technique was designed to prevent reflux of intestinal contents into the duct, and in healthy dogs proved remarkably effective, in clinical surgery however it is rarely if ever applicable to reconstruction of the biliary tract because there is not a sufficient length of bile duct available

Whipple (1941, 1946b), and Trimble, Parsons and Sherman (1941), anastomosed the common duct to the jejunum after one stage radical pancreaticoduodenectomy, and many surgeons have followed their example, some using a simple loop of jejunum and others a Y loop (for review *see* Brunschwig, 1942, Cattell and Warren, 1953)

Use in Present Day Surgery

Anastomosis of the common bile duct to the duodenum, by bypassing the sphincter of Oddi, allows reflux of duodenal contents into the bile duct, with consequent risk of cholangitis and hepatitis The risk is less if the duct is anastomosed to a simple loop of jejunum the limbs of which are connected by enteroanastomosis, and less still if the duct is anastomosed to a long Y loop these procedures however are more complicated and take longer to perform

In considering the indications for these three forms of choledocho enterostomy* the suitability and relative simplicity of the procedure must be balanced against the risk of ascending infection

The most clear-cut indication for choledochoduodenostomy as a short circuiting procedure is congenital atresia involving only the lower end of the *common bile duct*, but such cases are uncommon (Holmes, 1916, Ladd, 1935 Gross 1953) The operation gives better results than cholecystenterostomy, and because it can be performed more quickly is much safer in these seriously ill infants than either of the acceptable forms of choledochojejunostomy

*The patient already had a posterior gastrojejunostomy

*This term includes choledochoduodenostomy and choledochojejunostomy

Choledochoduodenostomy is used also by some surgeons to relieve obstructive jaundice due to chronic pancreatitis.

Choledocho-enterostomy has been used in stricture of the common bile duct due to inflammation or injury (Sanders, 1946), but is seldom indicated because if this procedure is feasible it is usually possible, after adequate mobilization, to resect the stricture and perform end-to-end anastomosis of the duct (*see* e g. Cattell, 1943a, Lahey and Pyrtek, 1950), thus conserving the sphincter of Oddi.

Choledocho-enterostomy is also rarely advisable as a short-circuiting procedure in patients with carcinoma of the head of the pancreas or the ampulla because the anastomosis would be placed too close to the tumour, and it is better in such cases to perform cholecystjejunostomy

On the other hand choledochojejunostomy is the standard method of reconstruction after pancreaticoduodenectomy Some surgeons use a simple loop of jejunum and anastomose the afferent and efferent limbs, others use a long Y-loop

ANASTOMOSIS OF THE HEPATIC DUCTS TO THE GASTRO-INTESTINAL TRACT

History

Direct anastomosis of the common hepatic duct to the duodenum for relief of obstruction due to a stricture appears to have been performed for the first time by W. J. Mayo (1905), and his patient was alive and well eighteen years later (Mayo, 1923). Further successful cases were reported by Judd and Burden (1924), Judd (1926), Walters (1926, 1932), Whipple (1927), Guerry (1930), and others, and by 1936 Eliot found a total of 56 cases recorded in the literature. This operation has since been used by many surgeons (*see* e g. Flickinger and Masson, 1946) Warren Cole, however, who has had a wide experience of the treatment of stricture of the bile ducts, states that he has had such poor results with the operation that he has abandoned it (Cole, Reynolds and Ireneus, 1948).

So-called indirect anastomosis, in which the duct is connected to the duodenum by a rubber tube, appears to have been first used by Jenckel (1905), although it is often attributed to Sullivan (1909), who improved the technique by wrapping omentum around the tube. Despite some apparently successful cases (*see* Jenckel, 1905; Brewer, 1910; Sullivan, 1912; Wilms, 1912; Eliot, 1936) the results, as might be expected, were generally unsatisfactory, and the procedure was abandoned.

Eliot (1918) cited three cases in which the common hepatic duct was anastomosed to the stomach, and further operations of the same kind were reported later by Tschassownikoff (1924) and Walzel (1930). Hoag (1937) obtained a good result in a single case by constructing a tube from the gastric mucosa and anastomosing it to the common hepatic duct over a rubber tube.

Anastomosis of the common hepatic duct to a Y-loop of jejunum was reported by Dahl (1909) and Lanphear (1909). This procedure, with various modifications in technique, has been used extensively in recent years in cases where end-to-end anastomosis of the duct appeared impossible. Allen (1945) splinted the anastomosis with a rubber tube and brought the lower end out through the wall of the jejunum and the abdominal wall. Warren Cole and his colleagues (Cole, Ireneus and Reynolds, 1945; Cole, Reynolds and Ireneus, 1948; Cole, 1948), Pearse (1946) and others used a vitallium tube* and left it in place permanently, but although some excellent results have been reported (Pearse, 1946; Cole *et al.*, 1948) this technique has been abandoned

*Vitallium tubes were first employed in reconstruction of the bile duct by Pearse (1941), who used a flanged vitallium tube as a splint after end-to end anastomosis of the duct.

owing to the risk of the tube becoming blocked (*see* Lahey, 1946 Cole, 1949) To obtain complete union of mucosa to mucosa in patients with only a very short stump of duct buried in scar tissue in the porta hepatis Cole (1948) has modified Hoag's (1937) operation A Roux loop is fashioned in the usual way and the end of the arm of jejunum is then trimmed so that the last inch consists only of mucosa After the scar tissue has been excised the mucosal sleeve is anastomosed to the duct over a rubber tube (Fig 180)

Anastomosis of the hepatic duct to a simple loop of jejunum has been found to carry a considerable risk of suppurative cholangitis even when entero anastomosis is performed (Cole *et al*, 1945), and this com

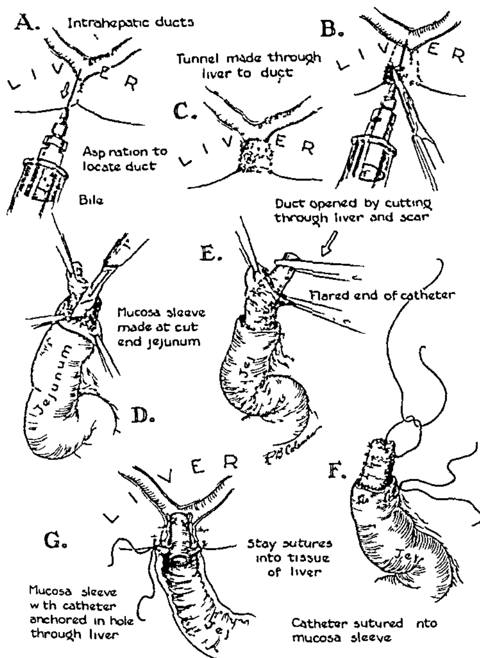


Fig 180 Warren Cole's modification of Hoag's operation for anastomosing a Y loop of jejunum to a very short stump of common hepatic duct buried in scar tissue in the porta hepatis (Reproduced from the *Canadian Medical Association Journal* by courtesy of the publishers and Dr Warren Cole)

plication may develop as late as two years after operation. The risk is lessened if three folds are fashioned in the afferent limb (Cole *et al.*, 1945, Peterson and Cole, 1948), but it is safer to use a long Roux loop. Cole for a time fashioned folds even when using a Roux loop, but later found that this was unnecessary provided that the loop* was at least sixteen inches long (Peterson and Cole, 1948).

A novel procedure was used by Falconer (personal communication) in a patient with a carcinoma at the upper end of the common hepatic duct involving the points of entrance of both the right and left hepatic ducts The right hepatic duct was anastomosed to the gall bladder and the gall bladder to the jejunum, thus permitting drainage of bile from the right lobe of the liver

Use in Present Day Surgery

In high traumatic strictures with only a short stump of normal common hepatic duct the best procedure, if end-to-end anastomosis of the hepatic to the common bile duct is impossible, is to anastomose the stump to a jejunal Roux loop The ascending limb of the loop should be at least sixteen inches long. Anastomosis to the duodenum is less satisfactory because of the danger of subsequent cholangitis

On the other hand in congenital atresia, if the only part of the biliary tract available for anastomosis is a nubble of hepatic duct, anastomosis to the duodenum is indicated because it is then the simplest and quickest procedure available and carries the lowest operative mortality.

ANASTOMOSIS OF BILIARY FISTULAE TO THE GASTRO-INTESTINAL TRACT

History

Many surgeons, including von Stubenrauch (1906), Sutton (1910), Lilienthal (1923), Lahey (1923, 1929), Dobrotworski (1925) and Russell (1933) have attempted to dissect out biliary fistulae with some surrounding tissue and anastomose them to the stomach, duodenum or jejunum. A somewhat different procedure was used by Hildebrandt (1922), who fashioned a Y-loop of jejunum, brought it out through the abdominal wall, and anastomosed it to the orifice of a biliary fistula. His patient, according to Eliot (1936), lived for five years, but the results on the whole have been poor (*see* Eliot, 1936 for review).

Use in Present Day Surgery

Anastomosis of biliary fistulae to the gastro-intestinal tract has proved to be an unreliable procedure and is now obsolete.

ANASTOMOSIS OF INTRA-HEPATIC DUCTS TO THE GASTRO-INTESTINAL TRACT

History

Baudouin (1896) and Langenbuch (1897) suggested that when no simple procedure was available it might be worth trying to establish communication between intrahepatic ducts and the gastro-intestinal tract by anastomosing a loop of jejunum to the margins of a defect created in the liver. An operation of this kind, which was apparently successful, was performed by Garrè (1908): prior to this unsuccessful operations had been reported by Kehr (1904) and Maylard (1905). Further attempts met with little success (*see* Redell, 1940, p. 33, for review), and the operation was abandoned. In 1948, however, Longmire and Sanford described a new operation in which they exposed a dilated intrahepatic duct by resecting a wedge of tissue from the left lobe of the liver, and made a mucosa-to-mucosa anastomosis between this duct and a loop of jejunum by the technique shown in Fig. 181. They performed this operation successfully

*The direction of peristalsis is away from the liver.

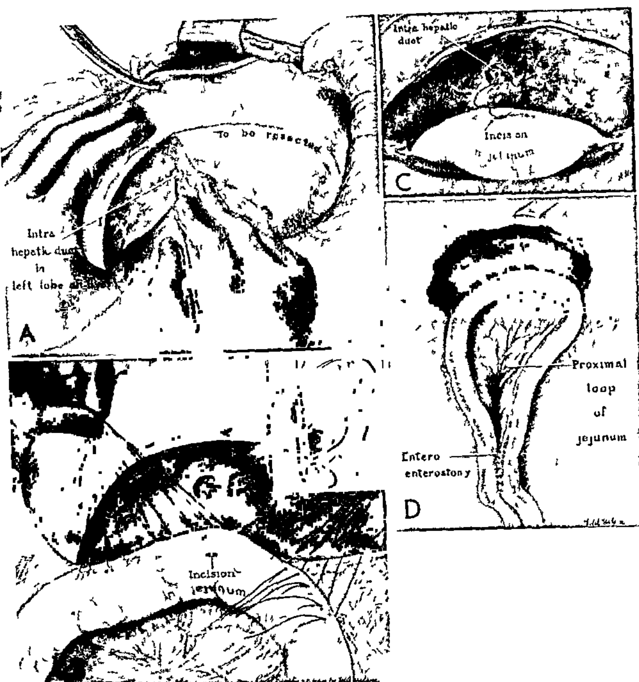


Fig 181 Anastomosis of an intra hepatic duct to a jejunal loop by the technique of Longmire and Sanford A A dilated duct is exposed by resecting part of the left lobes of the liver Deeply placed mattress sutures control bleeding B C A posterior row of interrupted sutures passing through the seromuscular coats of the jejunum and the capsule of the liver is inserted to anchor the loop to the liver an intestinal clamp is applied and a small opening is made in the jejunum in preparation for the anastomosis D The anastomosis between the duct and the jejunum has been completed a row of sutures has been inserted to anchor the loop to the capsule of the liver anteriorly and an anastomosis has been made between the afferent and efferent limbs of the loop (Reproduced from *Surgery* by courtesy of the publishers and Dr W B Longmire)

in three adults with non-malignant strictures of the bile ducts, and stated that they had in addition attempted it unsuccessfully in three infants with congenital obliteration of the bile ducts.

It has been suggested (Torrance, 1957; Holaday, 1958) that the main duct of the quadrate lobe may offer a suitable and accessible duct for anastomosis to a loop of jejunum in patients with obstructive jaundice due to lesions at the porta hepatis.

Use in Present Day Surgery

In cases of biliary obstruction due to congenital atresia or traumatic stricture in which no extra-hepatic part of the biliary tract is available for anastomosis, an attempt should be made to find an intrahepatic duct of adequate calibre and anastomose it to a jejunal loop as described above. In congenital atresia the chances of success are small, because in the first place the intrahepatic ducts may also be atretic, and secondly, the operation if feasible is time-consuming and carries a high mortality in a seriously ill infant.

In traumatic stricture the procedure should seldom be required, but when it is the chances of success are fairly good in skilful hands.

ANASTOMOSIS BETWEEN THE PANCREATIC DUCT AND THE GASTRO-INTESTINAL TRACT

History

Halsted (1899) succeeded in re-establishing connexion between the pancreatic duct and the duodenum after resecting the head of the pancreas and a wedge of duodenum, and some years later Kausch (1909b) successfully transplanted the cut end of the pancreas into the stump of the duodenum after a more extensive pancreaticoduodenectomy. Morian (1909), Oppenheimer (1912), and later various other surgeons, achieved the same thing after the simpler operation of transduodenal resection of a carcinoma of the ampulla of Vater.* Desjardins (1907) designed but did not perform, a two-stage radical pancreaticoduodenectomy in which he proposed to connect the stump of the pancreas to the jejunum, but Sauve (1908) felt that the danger of leakage would be too great and advised instead fixing the stump in the abdominal wall, thus creating a pancreatic fistula.

The obvious disadvantages of Sauve's procedure, and the fear of leakage if Desjardin's or Kausch's technique were used, deterred many surgeons from attempting to resect tumours of the ampulla or the pancreas, but stimulated Coffey to investigate the problem of pancreatico-enterostomy experimentally in dogs. As a result of his investigations Coffey (1909) evolved the technique illustrated in Figure 182, and showed that it could be used successfully to connect the stump of the pancreas to the jejunum after resecting the head, or alternatively to connect the tail of the pancreas* to the jejunum in order to short-circuit an obstruction at the ampulla. He suggested that, despite some anatomical differences, the procedure could be used in man, and described in detail a technique for radical pancreaticoduodenectomy, though he did not actually perform this operation clinically. Coffey also attempted in dogs to make a direct anastomosis between the main pancreatic duct and the jejunum, but without much success.

Sweet and Simons (1915), in a small num-

* In cases of the early transduodenal operations for carcinoma of the ampulla the pancreatic duct was apparently not identified. Both the successful and the unsuccessful operations have been reviewed by Upcott (1912), Outerbridge (1913), Cohen and Colp (1927), Hunt and Budd (1935) and Hunt (1941).

* After transecting it near the tip.

ber of dogs succeeded in connecting the pancreas to the small bowel by the much simpler technique illustrated in Figure 183 and an account of an essentially similar procedure was published two years later by Pratt, Pyle and Vale (1917) Tripodi and Sherwin (1934), also working with dogs divided the pancreas and connected the stump to the stomach through an incision in its posterior wall and their technique was subsequently modified by Person and Glenn (1939)

In the meantime two additional cases were reported one by Hirschel (1914) and the other by Tenani (1922), in which pancreaticoduodenectomy was performed successfully and in both the pancreas was reconnected to the remaining portion of the duodenum Most surgeons however continued to be deterred by fear of leakage of pancreatic secretion Whipple and his colleagues (Whipple Parsons and Mullins 1935 Whipple 1941) whose work revived widespread interest in pancreaticoduodenectomy Brunschwig (1937) and Trimble Parsons and Sherman (1941) dealt with

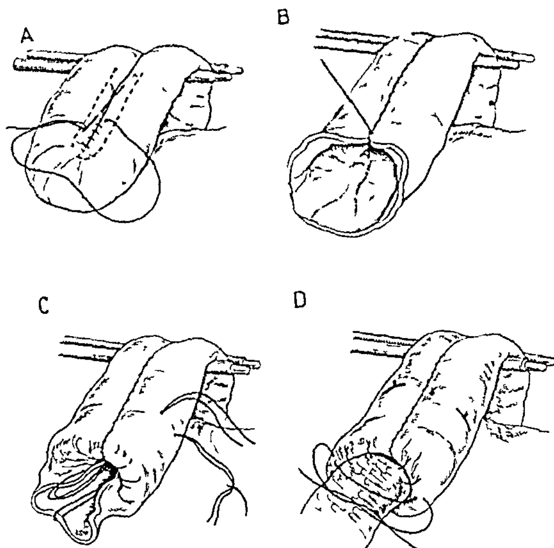


Fig 182 Coffey's technique of pancreaticojejunostomy A The two limbs of a loop of jejunum are united by a continuous seromuscular stitch after which the lumen is opened by an incision in the position of the dotted line B Sutures [illegible]



Fig. 183 Sweet and Simons' technique of pancreaticojejunal anastomosis. (Reproduced from *Annals of Surgery* by courtesy of the publishers.)

this problem by ligating the stump of the pancreas, and proved that patients could survive in the absence of the external secretion of the pancreas. Nemenyi (1937), on the other hand, successfully transplanted the stump of the pancreas into the open end of the duodenum after the manner of Kausch (p. 586), and Hunt (1941) in one case used a similar technique except that he removed the whole of the duodenum and therefore transplanted the stump of the pancreas into the open end of the jejunum.

There was for a time a difference of opinion as to which was safer: to ligate the pancreas or to reconnect it to the gastro-intestinal tract. Cattell and his colleagues (Cattell, 1943b, 1944, 1947, 1948; Cattell and Pyrtek, 1949; Cattell and Warren, 1953) consistently advocated and practised pancreaticojejunostomy, maintaining that the incidence of such complications as pancreatic fistula, peritonitis and post-operative haemorrhage was thereby reduced and the patient's nutrition much improved. Similar views were expressed by Orr (1945), and eventually Whipple (1946b) himself also became an advocate of pancreaticojejunostomy.

The technique used for the anastomosis by different surgeons differed in detail. Cattell (1943b) at first used a "necrosing suture" method (Fig. 184), similar to the Coffey III technique for ureterosigmoid anastomosis (p. 596); later (Cattell, 1948), however, he closed the stump of the pancreas except for the main duct with mattress sutures and anastomosed the duct to the jejunum over a rubber tube with two layers of sutures (Fig. 185).

Once a simple and safe technique for pancreaticojejunostomy was established it was natural to ask whether this could be used with advantage as a palliative procedure to improve the nutrition of patients with inoperable carcinoma of the ampulla or of the head of the pancreas.

Cattell (1947) showed that it could, and perfected a technique of side-to side anastomosis using a T-tube. He considered using the same procedure in selected cases of

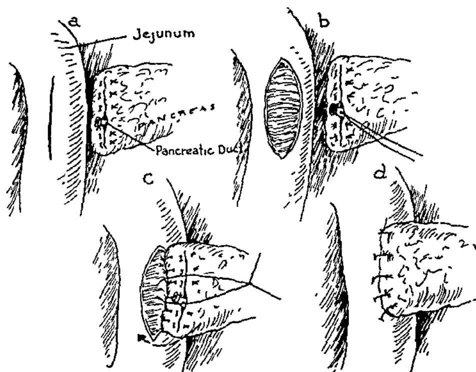


Fig 184 The necrosing suture method of pancreaticojejunal anastomosis a An incision has been made through the serosal and muscular coats of the jejunum Sutures have been inserted in the cut end of the pancreas and the pancreatic duct has been ligated b The margins of the incision in the jejunum have been separated exposing the mucosa The pancreatic duct has been transfixed by a silk suture c The posterior surface of the pancreas has been joined to the lateral wall of the jejunum and the transfixing silk suture now transfixes the mucosa of the jejunum d The anastomosis has been completed by inserting an anterior row of sutures between pancreas and jejunum (Reproduced from *Surgical Clinics of North America* by courtesy of the publishers and Dr R B Cattell)

chronic relapsing pancreatitis but concluded that it would seldom be justified (Cattell and Warren, 1953) Hay (1950) actually divided the main pancreatic duct and anastomosed it end to side to the duodenum in three patients with intractable chronic pancreatitis, but such a formidable procedure, as Cattell and Warren (1953) have pointed out would be even less *justified*

More recently excision of the tail, or tail and part of the body, of the pancreas, combined with retrograde pancreaticojejunostomy has been used to deal with chronic pancreatitis associated with obstruction of the duct by strictures or calculi (Zollinger Keith and Ellison, 1954; Aird and Buckwalter, 1955, Longmire, Jordan and Briggs, 1956 DuVal, 1957) The pancreatic duct system may also be anastomosed to a Y-loop of jejunum after division of the neck of the pancreas When there are multiple strictures of the pancreatic duct system, as in some cases of chronic pancreatitis, Puestow (Puestow and Gillesby, 1958) opens the pancreatic duct throughout the whole length of the neck and body of the pancreas and re establishes drainage of pancreatic secretion by anastomosing a Y loop of jejunum to the anterior surface of the pancreas

Use in Present Day Surgery

Pancreaticoduodenectomy is the opera-

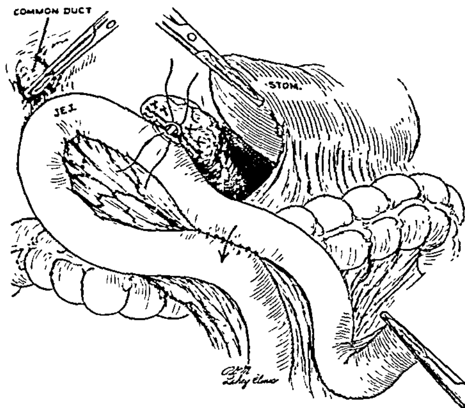


Fig 185 Pancreaticojejunostomy using a two-layer anastomosis. (Reproduced from *Surgical Clinics of North America* by courtesy of the publishers and Dr. R B. Cattell)

tion of choice in carcinoma of the ampulla of Vater when the tumour has not spread locally beyond the possible limits of resection, does not involve the superior mesenteric vessels or portal vein, and has not given rise to distant metastases. It is, in addition, still performed occasionally for carcinoma of the head of the pancreas, but the results are poor and it is usually better to be content with a palliative procedure.

Pancreaticoduodenectomy is also indicated sometimes in benign stricture of the pancreatic duct, and very occasionally in cystadenoma of the pancreas, and in islet-cell adenoma when the tumour cannot be found.

Whenever pancreaticoduodenectomy is performed the main pancreatic duct should be anastomosed to the jejunum, preferably by the technique illustrated in Figure 185.

In inoperable cancer of the ampulla and head of the pancreas cholecystjejunostomy (p. 580) is the usual palliative procedure. It would seem logical in addition to anastomose the pancreatic duct to the jejunum as advocated by Cattell, but few other surgeons appear to have done this.

CHAPTER 28

Pedicle Transplantation and Anastomosis of Hollow Viscera
II. Reconstruction of the Urinary Tract

INTRODUCTION

The operations to be considered are of two main types. In the first type, termed *diversion operations*, the ureters are divided and anastomosed to the colon, or to an isolated loop of bowel which opens on the surface of the body.* In the second type, termed by Pyrah (1957) *remodelling operations*, a segment of ileum is used to replace part or the whole of a ureter or the urinary bladder, or to increase the capacity of the bladder, and the patient continues to pass urine *per urethram*.

HISTORY

DIVERSION OPERATIONS

Urinary diversion operations involving the use of colon or ileum may be classified as follows:

1. Anastomosis of the ureters to the colon including the rectum (ureterocolostomy) without interrupting the continuity of the bowel.

2. Anastomosis of the ureters to a *partially isolated segment of colon*.

3. Anastomosis of the ureters to a *completely isolated segment of bowel*.
   (1) *Ileal "bladder"*
   (2) *Ileocaecal "bladder"*
   (3) Rectosigmoid "bladder"

The development of these operations has proved a difficult task, the main hazards to be overcome being (a) leakage at the anastomosis, (b) ureteral obstruction, (c) ascending infection of the kidney, and (d) metabolic disturbances. The technical devices which have been tried with the object of preventing leakage, obstruction and ascending infection will be considered in discussing the various types of operation. The metabolic disturbances resulting from diversion of urine to the bowel are of interest to biochemists and physiologists as well as to surgeons, and will be considered in a separate section (p. 607).

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*The ureters have also been made to open on the skin (cutaneous ureterostomy) or into the vagina. Cutaneous ureterostomy was introduced by Vasil'ev (1895) and favourable reports have been published from time to time (e.g. Cabot and Scherer 1935, Keyes 1940, 1943, Folsom and O'Brien 1943, Mathé 1945, *Trabucco and* Marquez 1950). It has however never been very popular largely because of the high incidence of stricture formation (see e.g. Annis, Hunter and Wells 1954) and is becoming—if indeed it is not already—obsolete. Anastomosis to the vagina, which was used first by Pawlik (1891) and Robson (1902) is completely obsolete. Another procedure which is currently used in reconstruc*tion of the urinary tract is to re-implant one or both* ureters in the bladder. From the point of view of the present book this procedure is of interest mainly in relation to transplantation of the kidney, and it has already been considered in that connexion (p. 53?).

Ureterocolostomy without Interruption of the Continuity of the Bowel

From 1851 to 1911

In July, 1851, John Simon of St. Thomas' Hospital attempted to divert the urine to the rectum in a boy with ectopia vesicae (Simon, 1852) Under chloroform anaesthesia he passed a metal catheter up one ureter, inserted a stilette into the catheter and on into the rectum, and pulled through a thread. He passed a second thread from the ureter to the rectum in the same way at a slightly lower level, knotted the rectal ends of the two threads together, and then pulled out the second thread so that the first formed a loop passing from the ureter to the rectum and back to the ureter. He repeated the procedure with the opposite ureter, and by gradually pulling on the threads succeeded in creating uretero-rectal fistulae. The boy lived for a year after this ingenious operation, and passed much of his urine *per rectum* He suffered from repeated attacks of fever, however, and died with pelvic peritonitis At autopsy "incipient calculous formation" was observed in each ureter Lloyd (cited by Kirwin, 1934) attempted a similar operation in October, 1851, but his patient died on the seventh day from peritonitis

The next recorded attempt to treat a patient with ectopia vesicae by diverting the urine was made more than a quarter of a century later by Thomas Smith (1879) of St. Bartholomew's Hospital, who transplanted the left ureter extraperitoneally to the descending colon by direct end-to-side mucosa-to-mucosa anastomosis and fourteen months later transplanted the right ureter to the ascending colon by the same technique. The patient developed a fistula in the loin after the first operation, but this gradually closed. Three days after the second operation, however, he died, and at autopsy the left kidney was found to consist almost entirely of fibrous tissue.

The experimental approach to the problem was initiated by Gluck and Zeller (1881). They removed the bladder of a dog and inserted the ends of the ureters into the rectum through simple stab wounds, but the animal died of peritonitis.* Further experiments in which one or both ureters were anastomosed to the colon or rectum were reported by Bardenheuer (1886), Tuffier (1888). Reed (1892), Morestin (1893), van Hook (1893), Thomson (1893), Giordano (1894), Chaput (1894), Lindner (1895), Vignoni (1895), Boari (1895, 1896), Krynski (1896), Tuffier and Dujarier (1898), Frank (1899), Kalabin (1899a, b), Martin (1899a, b), Duval and Tesson (1900), Connell (1901), Peterson (1901), Druebert (1902) and others.

Many different techniques were used, some of which have been re-introduced later, but the results on the whole were very poor and the mortality was high.

Bardenheuer (1886) and Morestin (1893) buried the distal part of the ureter by inserting sero-muscular sutures after the manner of Witzel's (1891) gastrostomy. Nearly all their animals died, but this procedure, with various modifications was subsequently popularized by Stiles (p. 594) and given extensive clinical trial.

Giordano (1894) in one dog inserted a tube into each ureter and brought the ends of the tubes out through the anus. The animal died soon after the operation but a similar manoeuvre was used subsequently by Coffey in his second technique (p. 595). Vignoni (1895) fashioned a V-shaped flap from the whole thickness of the rectal wall to act as a valve but failed to prevent ascending infection of the kidney.

Boari (1895) anastomosed one ureter to the colon by means of a special button in four dogs, and all survived the operation; as the animals were killed within a few weeks, however, the effectiveness of the

*Another animal, in which Gluck and Zeller performed cystectomy and cutaneous ureterostomy, survived

method in permanently preventing ascending infection cannot be assessed

Krynski (1896) obtained good results in dogs with a new technique, similar in principle of Coffey's first technique (p 594), in which he dissected up a triangular seromuscular flap from the wall of the rectum, passed the end of the divided ureter through a small incision in the mucosa at the lower angle of the triangle, and sutured the flap back in position Martin (1899a, b) also had some success with another technique in which the ureter for a short distance proximal to its point of entry into the lumen of the bowel, was buried beneath the serosa, but Duval and Tesson (1900) who used a technique very similar to that of Krynski, were unable to prevent ascending infection of the kidney

Peterson (1901) and Drucbert (1902) transplanted a portion of bladder wall with both ureteric orifices to the rectum—a procedure used clinically some years later by Maydl (*v infra*)—but found that ascending infection still occurred It is noteworthy however that one of Drucbert's animals lived for more than a year before it died of pyelonephritis

Steinke (1909) reviewing all this experimental work found that out of 134 dogs in which bilateral ureteric transplantation was reported to have been performed there were 117 deaths due to peritonitis pyelonephritis or both, and of the 17 survivors 9 eventually developed pyelonephritis on one or both sides

In the clinical field Maydl (1894) transplanted part of the bladder wall with both ureteric orifices to the pelvic colon in a patient with ectopia vesicae The operation was successful and Maydl (1896 1899) subsequently treated nine other patients in the same way This operation with various modifications (*see* e g Moynihan 1906) enjoyed a considerable vogue and by 1909 Buchanan collected 80 cases in which it had been performed, of whom 23 had died as an immediate result of the operation In the same year Zesas (1909) collected 97 cases, of whom 71 had survived He reported that fatal ascending infection sometimes occurred as long as two years after operation

Bergenhem (1895) in Sweden treated a patient with ectopia by transplanting each ureter to the rectum with a surrounding rosette of bladder wall This operation is often attributed to Peters (1901) of Toronto, but according to Buchanan (1909), Peters was preceded not only by Bergenhem but also by surgeons in Italy the United States and Australia

Maydl's and Bergenhem's operations were devised in the hope that the ureteric orifice would prevent ascending infection, but in fact the normal valve like action of the orifice was lost after transplantation Another approach to the problem was adopted by Fowler (1898) who transplanted the ureters to the rectum in a patient with ectopia in such a way that each orifice was guarded by a valve like flap of mucosa and his patient was reported to be alive and well three months later Three other patients in whom this operation was performed, however, died shortly after operation from ascending infection or ureteric obstruction

In a few cases the ureters were successfully transplanted to the rectum in patients with carcinoma of the bladder, notably by Chalot (1896) Tuffier and Dujarier (1898), von Winiwarter (1898) and Krause (1899) Chalot's case is especially remarkable in that he performed total cystectomy and hysterectomy and his patient was alive and well a year later There were however many deaths (Kuster 1891, Lund, 1902 Woolsey, 1903 Watson 1905, for review) some from shock in the immediate post operative period and others—as in Woolsey's case—at a later stage from pyelonephritis

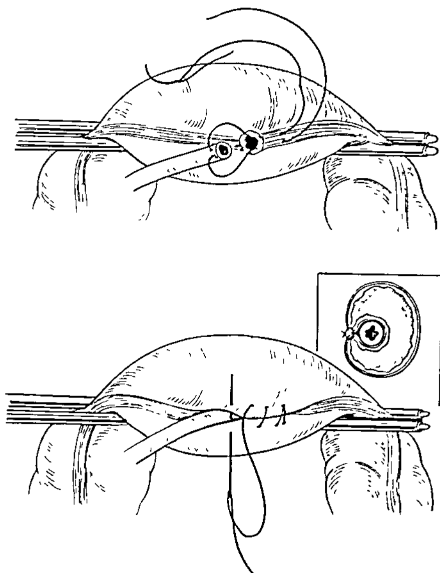


Fig 186 Ureterocolostomy Stiles' technique.

From 1911 to the Present Day

In 1911 Stiles of Edinburgh reported that he had successfully performed ureterocolostomy in two girls with urinary incontinence due to epispadias* by drawing the end of the divided ureter into the colon through a small incision and then burying a short segment of ureter proximal to the point of entry by means of a seromuscular suture (Fig. 186). This method has since been used extensively (*see* e.g. Grey Turner, 1929), and in English speaking countries is usually known as Stiles' technique.

*Stiles' first patient was operated on in 1907

In the same year an American surgeon, R. C. Coffey, described another technique, later known as the Coffey I technique, which has also been widely used. In this method (Fig. 187), which is similar to that used previously by Coffey for choledochojejunostomy (p. 581), an incision about 3 cm. long is made through the serous and muscular coats of the colon exposing the mucosa. A small stab wound is made through the mucosa at the lower end of the incision and the proximal stump of the divided ureter is drawn through this opening into the colon by means of a traction suture. The ends of

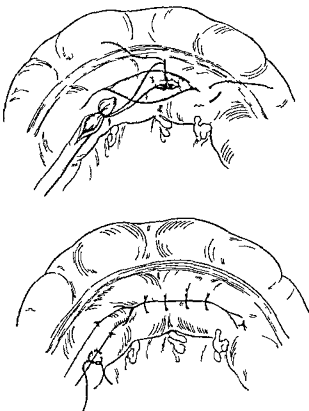


Fig 187 Ureterocolostomy The Coffey I technique

this suture are brought out again through the wall of the colon and tied after which sero muscular sutures are inserted to bury the lower part of the ureter between the muscle and the mucosa of the colon Coffey (1911) found in experiments in dogs that the incidence of ascending infection was reduced by using this technique and that if the pelvic colon was isolated after death and distended with fluid *none of the fluid entered the ureters* According to Coffey (1931) this procedure was first used in a patient by C H Mayo Coffey himself first used it in a patient in October 1913

It soon became apparent that while this technique might reduce the incidence of *ascending infection it certainly did not* abolish it It was generally accepted that micro-organisms reached the kidney via the lumen of the ureter though as early as 1909 Steinke had suggested that they might pass upwards in the peri ureteral lymphatics Sweet and Stewart (1914) investigated the matter and concluded that Steinke s suggestion was correct Among other ingenious experiments they transplanted the ureter without interrupting its continuity in such a way that for part of its course it lay within the lumen of the colon and found that ascending infection still occurred

Heitz Boyer and Hovelacque (1912) suggested that the incidence of ascending infection and incidentally of leakage at the anastomosis might be reduced by performing nephrostomy prior to the ureteric transplantation This procedure was reintroduced by Hinman (1926b) who used it mainly in patients with tuberculous cystitis

In 1925 Coffey described another procedure now known as the Coffey II technique differing from the Coffey I in that the anastomosis is splinted by a rubber tube or ureteric catheter which is brought out through the anus and usually remains in place for about a fortnight In later papers (Coffey 1927 1928 1929 1930b) he described minor modifications of this procedure and reported on its clinical use The Coffey II technique was devised primarily to deal with the problem of anastomosing dilated ureters to the colon but Coffey soon decided that its main advantage was that it provided a relatively safe method of transplanting both ureters in one operation a *procedure which had previously been re*garded as unjustified owing to the risk that both anastomoses might fail to function He used it at first in the palliative treatment of carcinoma of the bladder and in the treatment of non malignant conditions such as ectopia vesicae but in 1930 reported a case *in which it was used successfully in con*junction with total cystectomy for bladder carcinoma (Coffey 1930b)

*The Coffey II technique has been modi*fied by various other surgeons C H Mayo (Mayo 1919 Mayo and Hendricks 1926) used a piece of catgut instead of a rub

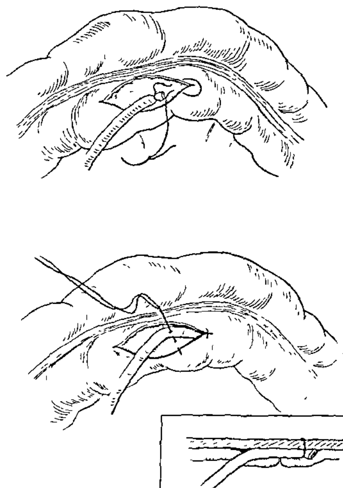


Fig 188 Ureterocolostomy The Coffey III (necrosing suture) technique

ber tube or ureteric catheter to splint the anastomosis, but transplanted only one ureter at a time. Furniss (1932) used catheters and passed them into the colon on a special trocar to prevent soiling. Nitch (1932) passed the catheters into a lead tube inserted through the anus by an assistant and guided by the operator to the level required Green-Armytage (1932) used a similar technique except that he employed a cystoscope instead of the lead tube.

Coffey (1930a, b, c) described yet another technique, the Coffey III, in which the end of the ureter is ligated and a ureterocolic fistula is created in the course of a few days by means of a tightly tied necrosing suture which transfixes both the ureter and the mucosa of the colon (Fig. 188). Coffey obtained excellent results with this technique in dogs, and in 1931 reported one clinical case in which he had used it successfully. He pointed out that only one ureter could be transplanted at a time* but predicted that the technique would nevertheless be widely used in the treatment of ectopia vesicae on account of its safety and simplicity; this prediction, however, has not been fulfilled.

Further experiments in dogs were performed by many investigators. Sisk, Weir

*Hinman (quoted Hinman and Weyrauch, 1937) transplanted both ureters simultaneously in three patients by a modified Coffey III technique in which he drained each ureter by a catheter brought out extraperitoneally, but one patient died, and in another patient one kidney had to be removed on account of ascending infection

and O Brien (1931) compared the Coffey II and Mayo techniques, and found that the former gave the better results though neither proved very successful Ferguson (1931) embedded about 3 cm of each ureter in the wall of the colon without interrupting its continuity At a second operation he divided the ureters distal to their point of emergence from the bowel wall, passed a diathermy electrode up each ureter in turn and made an opening between the ureter and the colon by fulguration and after withdrawing the electrode ligated the end of the ureter and buried it in the wall of the colon Douglass and Edwards (1936) found that while the first stage of Ferguson s procedure was well tolerated the second often failed because of local infection or because the fistula failed to remain open, they modified the technique by passing an insulated wire loop round the intramural part of the ureter and out through the anus at the original operation with the object of completing the anastomosis by passing a high frequency current through the loop, but their experiments were not very successful Walker Taylor (1931) experimented with several techniques including his so called irreversible tunnel technique in which an intramural tunnel is prepared in the colon with a blunt dissector and the ureter is drawn into the bowel by means of a traction suture attached to a very long (nine inch) straight needle which pierces the mucosa at the distal end of the tunnel and is then brought out through a metal tube passed up through the anus Bolliger and Walker Taylor (1936) subsequently published a follow up report on one dog which survived for three years following ureterocolostomy by this technique and removal of the opposite kidney sixteen days later For two years the animal remained in excellent health though its blood urea was somewhat elevated thereafter no further observations were made until it died a year later with pyonephrosis and abscesses in the kidney

Higgins (1933 1934, 1935) modified the Coffey III technique by not dividing or occluding the ureters until the uretero sigmoid fistulae were established He used this procedure successfully first in dogs and later in patients Brenizer (1936) further modified the technique by passing the necrosing suture through a metal ring inserted into the rectum through the anus and by inserting an insulated wire after the manner of Douglass and Edwards (*v supra*) with which he divided the ureter after the ureterocolic fistula was established Wadhams and Carabba (1935) in a single experiment in a dog instead of using a necrosing suture treated small areas of the colonic mucosa and the wall of the ureter by electro coagulation prior to bringing them into apposition by non penetrating sutures Ureterocolic fistulae developed but obstruction and ascending infection occurred on one side

Beaver and Mann (1932) omitted the intramural tunnel altogether and inserted the ureters through simple stab wounds in the colon A similar technique was used later by Huggins and Johnson (1947) and Kerr and Colby (1949)

Zollinger (1934) performed ureterocolic anastomosis in dogs by means of a mechanical device made in two parts and resembling a dress snap (press stud) and obtained encouraging results but his procedure has gained little support and does not appear to have been used clinically

Kirwin (1934) recorded that in three years of experimenting he had not succeeded in obtaining a satisfactory uretero colic anastomosis in the dog but suggested that the problem might not be quite so intractable in man His paper is of special value because it incorporates a comprehensive review of previous work in this field Another valuable review was published by Hinman and Weyrauch (1937)

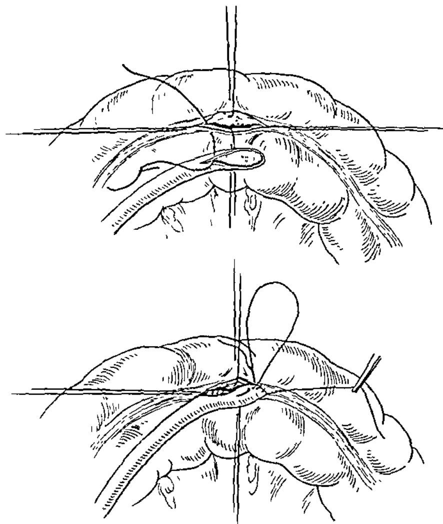


Fig 189. Ureterocolostomy by mucosa-to-mucosa suture Nesbit's technique.

Jewett (1910, 1912, 1944) described a two-stage procedure which he regarded as original but which differs only in detail from that of Ferguson (p. 597). After successful trials in dogs he used it with encouraging results in more than thirty patients.

Hinman and Weyrauch (1942) showed experimentally that strictures and fistulae were likely to develop after ureterocolostomy if infection or ischaemic necrosis occurred in the tip of the ureter. They found the Coffey III technique unsatisfactory, and obtained their best results with a modified Coffey I anastomosis in which the end of the ureter was bevelled, and its course in the wall of the bowel was made very short.

Brunschwig (1948), in his operation of pelvic evisceration for advanced carcinoma, anastomosed the ureters to the sigmoid colon proximal to the colostomy by a Coffey I technique and collected the urine in a Rutzen bag.

Schnittman (1948) modified the Coffey I technique by dissecting the muscle of the colon away from the mucosa for a distance of about 1.5 cm. on each side of the incision so as to form a wide trough. He claimed in this way to reduce the incidence of stricture formation.

Nesbit (1948, 1949) and Cordonnier (1949, 1950) introduced direct anastomosis of mucosa-to-mucosa by suture. These tech-

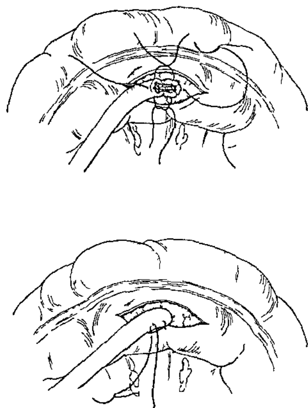


Fig 190 Ureterocolostomy by mucosa to mucosa suture Cordonnier s technique

niques (Figs 189 190) have since been used extensively but have the disadvantage of permitting extensive reflux of bowel contents (Whisenand and Moore 1951 Jacobs and Stirling 1952)

Leadbetter (1951) combined mucosa to mucosa suture with the use of a Coffey type tunnel (Fig 191) hoping thereby to avoid both stricture formation and reflux of bowel contents He performed this operation in eight patients and reported that the results ranged from good to excellent Weyrauch and Young (1952) used the same technique in dogs and reported that it gave better results than either the Coffey I Cordonnier or Higgins procedures Woodruff Cooper and Leadbetter (1952) in very similar experiments reached the same conclusion Later Leadbetter and Clarke (1955) reviewed the results obtained in 40 patients These were assessed as excellent in 24 patients and good in 7 others Three patients developed pyelonephritis and six developed a significant degree of hydronephrosis or hydroureter In another paper Clarke and Leadbetter (1955) reviewed the results of ureterosigmoidostomy in 2 897 cases reported in the literature They found that Leadbetter s technique gave the lowest incidence of ascending infection and hydronephrosis, and about the same incidence of metabolic disturbance (hyperchloraemic acidosis—*see* p 609) as the Coffey Stiles Nesbit and Cordonnier procedures A further refinement has been introduced by Weyrauch (1956) who uses a rubber catheter to splint each anastomosis The tip of each catheter is passed upwards along the corresponding ureter to the renal pelvis and its distal end is brought out through the anus by passing it down through a tube introduced into the rectum The catheters are usually removed after seven days

Goodwin Harris Kaufman and Beal (1953) in experiments in dogs used a more complicated combined technique (Fig 192) in which after dividing the ureter low down and attaching a traction suture to the end of the proximal portion they opened the sigmoid colon by a longitudinal incision in the anterior taenia incised the mucosa posteriorly pushed a forceps obliquely through the bowel wall into the retroperitoneal space grasped the traction suture and gently drew the end of the ureter into the bowel They then anastomosed the ureter to the colon under direct vision by interrupted chromic catgut sutures passing through the colonic mucosa and the whole thickness of the wall of the ureter

Yet another technique was used by Mathisen (1953) who sutured a full thickness flap of bowel wall around the terminal part of the ureter and invaginated it into the lumen so as to form a nipple like projection Like Goodwin *et al* he claimed that reflux did not occur

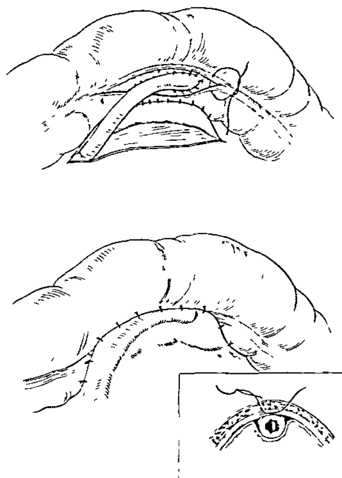


Fig 191 Ureterocolostomy by mucosa-to-mucosa suture combined with an intramural tunnel Leadbetter's technique.

Ureterocolostomy Using a Partially Isolated Segment of Colon

Borelius* (1903a, b), in two patients with ectopia vesicae, anastomosed the ureters to a loop of sigmoid by Maydl's method (p 593) and performed side-to-side anastomosis at the base of the loop. Von Misch (1907) modified this operation by placing a ligature around the colon just proximal to the ureteric anastomosis. Mueller (1903) suggested constructing a form of Y-loop by completely dividing the sigmoid and anastomosing the end of the proximal portion to the side of the distal, and then anastomosing the trigone to the end of the distal portion. This operation was performed successfully in a patient with ectopia by Floercken (1922). Muscatello (1905) and Dowden (1909) used a similar technique except that they closed the end of the proximal portion of bowel and restored continuity by side-to-side anastomosis.

*Acting on a suggestion made by his assistant, Berglund.

Anastomosis of the Ureters to an Isolated Pedicled Segment of Bowel†

The term *isolated pedicled segment of bowel*, or more briefly *isolated segment of bowel*, means a segment which retains its blood supply but no longer forms part of the intestinal tract. When such a segment is used as the terminal part of the urinary

†In preparing this section much help has been obtained from excellent reviews published by Hinman and Weyrauch (1936, 1937) and Weyrauch (1936).

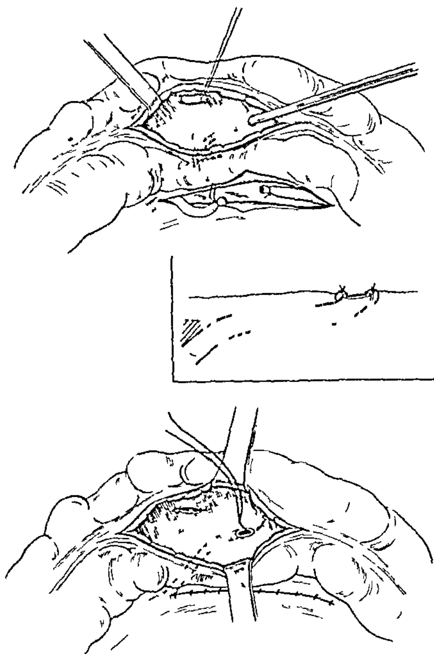


Fig 19 Ureterocolostomy by mucosa to mucosa suture combined with an intramural tunnel Technique of Goodwin Harris Kaufman and Beal

tract it is often referred to as a substitute bladder

Ileal "Bladder"

Cuneo (cited by Marion 1912) in two patients with ectopia vesicae isolated a segment of ileum closed one end and by a combined abdominal and perineal approach brought the other end to the perineum through the anal sphincter muscle anterior to the mucosa of the rectum Later he anastomosed the ureters to the ileal segment in one case by Bergenhem s and in the other by Maydl s technique (p 593) Both patients recovered from the operation but developed urinary fistulae Some years later a similar operation was performed by Kotzenberg (1917) but the patient died of peritonitis

Bricker (1950 1952) anastomosed the ure

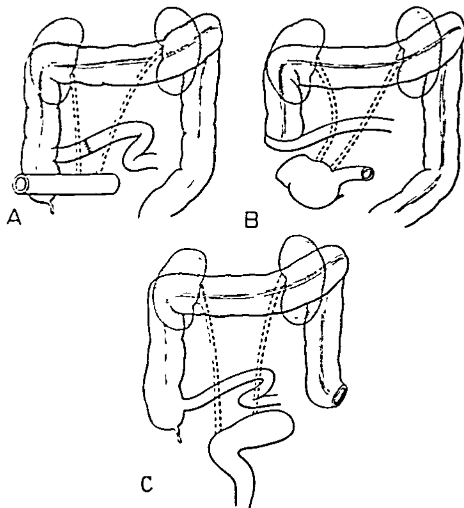


Fig. 193. Diagram illustrating methods of diverting of urine to an isolated segment of bowel. A. Ileal "bladder." B. Ileo-caecal "bladder." C. Recto-sigmoid "bladder."

ters to an isolated loop of ileum, one end of which was closed and the other brought to the surface as an ileostomy (Fig. 193A). Urine drained continually and was collected in a Rutzen bag. In 1952 he reported 55 patients treated in this way. Twenty-eight had been followed for six months or longer, and of these 17 showed no renal infection and little or no hyperchloraemic acidosis (p. 609), and only one showed an elevation of the blood non-protein nitrogen. A similar procedure has been used with good results by Wells and his colleagues (Wells, 1953; Annis, Hunter and Wells, 1953, 1954; Annis, 1956), Cordonnier (1955), Leadbetter and Shaffer (1956), Pyrah (1957) and other surgeons. The method has been studied experimentally in dogs by Irvine, Allen and Webster (1956).

Goldenberg (1904), Berg (1907), and Moskowicz (1909) anastomosed the ureters to a partly isolated loop of ileum emptying into some part of the large bowel, but this type of procedure has long been abandoned.

Ileocaecal "Bladder"

Anastomosis of the ureters to a completely isolated ileocaecal segment (Fig. 193B) was initiated by Verhoogen and De Graeuwe (De Graeuwe, 1908; Verhoogen, 1908; Verhoogen and De Graeuwe, 1909), who used the appendix as urethra. A similar tech

nique was used subsequently by Makkas (1910) von Fink (1910) Lengemann (1912) Taddei (1913) Freund (1916) Scheele (1923a) and Cortes (1946)

In 1950 Bricker and Eiseman reported a case in which they anastomosed the ureters to the caecum after isolating an ileocaecal segment and used the terminal ileum as urethra This procedure was later studied experimentally and also used clinically by Merricks and his colleagues (Gilchrist Merricks Hamlin and Rieger 1950 Merricks Gilchrist Hamlin and Rieger 1951 Merricks 1955) Further clinical cases in which it has been used have been reported by Peck and Newland (1952) Creevy and Reiser (1952) Glaser (1952) Wenger (1952) Rieger and Weisser (1952) Moore (1953) Robinson (1955) and Jay Borski and Kimbrough (1955) As a rule the patient has catheterized himself and the success of the procedure appears to have been influenced to a considerable extent by the frequency and regularity with which this manoeuvre was carried out

Rectosigmoid "Bladder"

Mauclaire (1895) performed sigmoid colostomy in dogs and anastomosed the ureters to the isolated lower sigmoid and rectum This operation (Fig 193C) was performed clinically for the first time by Remedi (1906) in a patient with ectopia vesicae The whole procedure was *completed in one stage but the patient died four days later* Soon afterwards Kronig (1907) performed a similar operation in two stages in a woman with a tumour of the uterus involving the bladder and the patient was reported to be alive and well five years later (Rubin 1918) Subsequently Latzko (1910) reported an unsuccessful operation and Pryr (1914b) two successful ones in patients with carcinoma of the bladder Further successful cases in which Remedi s operation was combined with total cystectomy for carcinoma of the bladder were recorded by Thies (1914) and Schmieden (1923) in Germany Kodis (1916) in Russia and Lindstrom (1928) in Scandinavia

More recently the procedure has been studied experimentally *in dogs by Higgins* (1948) and Irvine Allen and Webster (1956) and used clinically by Melick and his colleagues (Kinman Sauer Houston and Melick 1953 Melick and Naryka 1955) Gross Holcomb and Swan (1953) Pyrah (1954 1957) Paull and Hodges (1955) and Smith and Hinman (1955) The results generally speaking have been excellent none of the patients developing ascending infection after operation and very few showing evidence of any metabolic disturbance The absence of complications appears to be due partly to the low voiding pressure which according to Smith and Hinman (1955) is only about 30 cm water

In order to maintain continence of faeces as well as of *urine Gersuny (1899) trans*planted the trigone of the bladder to the isolated rectum by Maydl s method (p 593) and then brought the sigmoid colon down just anterior to the rectum but within the anal sphincter to the perineum This operation was modified by Heitz Boyer and Hovelacque (1912) who anastomosed the ureters separately to the isolated rectum instead of employing Maydl s technique and brought the sigmoid colon down posterior instead of anterior to the rectum A further modification was introduced by Lastaria (1913) who stripped the serosa and muscularis from the part of the sigmoid colon lying within the anal sphincter

Cases in which the operation of Heitz Boyer and Hovelacque or some minor modifications of it was performed were reported by Vulliet (1913) Gosset (1913) and Gregoire (1914) *More recently operations dif*fering only in detail from those we have been considering have been described by Mikuli (1930) Levitsky (1953) and Lowsley and his colleagues (Lowsley Johnson and Rueda 1953 Lowsley and Johnson 1955)

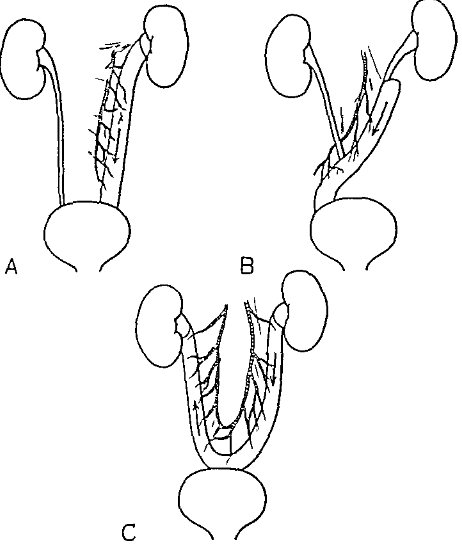


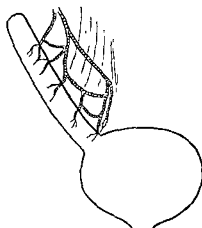
Fig 191 Methods of using a loop of ileum for reconstruction of the ureter The arrows indicate the direction of peristalsis Note that the flow of urine is isoperistaltic in A and B whereas in C it is isoperistaltic on the left side of the patient and antiperistaltic on the right

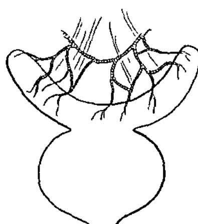
Lotheissen (1899) in experiments on dogs and cadavers anastomosed the ureters to a pouch constructed from the anterior rectal wall He thought this operation would be safer than that of Gersuny but the obvious difficulties of the procedure have prevented it from ever being used clinically

REMODELLING OPERATIONS USING AN ISOLATED PEDICLED SEGMENT OF BOWEL

Replacement or Reconstruction of the Ureter

Tizzoni and Foggi (1888), in experiments in dogs, isolated a segment of ileum, closed one end, and sutured the other end to the neck of the bladder Continuity of the bowel was restored by ileo ileal anastomosis At a second operation they anastomosed the ureters to the ileal segment They reported that sphincteric control of micturition was established Subsequently d Urso and de Fabii (1900) and Melnikoff (1912), also working with dogs, successfully used an isolated ileal segment to replace part of the ureter More recently the feasibility of this procedure has been confirmed in numerous experiments by Davids and Lesnick (1953), Annis (1953),


A


B

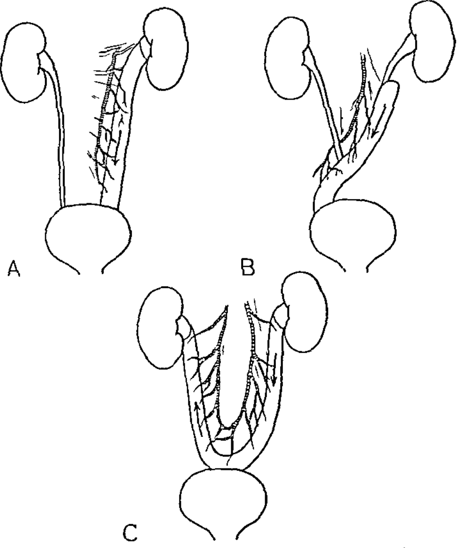


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this anastomosis broke down and the patient died eighteen days later.

More recently this type of procedure has been studied in dogs by Bisgard and his colleagues (Bisgard, 1943; Bisgard and Kerr, 1949), Rubin (1948, 1951) and Thompson (1950), who constructed a substitute bladder from an isolated pouch of sigmoid colon and anastomosed this to the urethra.

Couvelaire (1951), in a patient with carcinoma of the bladder, anastomosed the ureters to an isolated loop of ileum and, after performing total cystectomy, joined the distal end of the loop to the prostatic urethra and brought the proximal end out through the abdominal wall. Later he closed the fistula and the patient then passed all his urine *per urethram*. Micturition was produced by a combination of straining and manual pressure over the lower abdomen. A somewhat different technique was used more recently in two patients by Pyrah (1956, 1957), and in one patient by Raper (cited Pyrah, 1957), in which, following total cystectomy, the ureters were anastomosed to the ends of an ileal segment and the centre of the segment was anastomosed to the prostatic urethra (Fig. 196). A catheter was retained in the urethra for 10 days. Both of Pyrah's patients had cancer of the bladder. One died from pulmonary embolism four weeks after operation; the other made a good recovery and returned to work, but died two years later from recurrence of his tumour. Raper's patient was operated on because of recurrent Hunner's ulcer, and the operation appears to have been highly successful.

METABOLIC DISTURBANCES AFTER ANASTOMOSIS BETWEEN THE URINARY TRACT AND THE INTESTINE

Observations in Dogs

Ureteroduodenostomy

It was shown by Baird, Scott and Spender (1917) that dogs survived anastomosis of one ureter to the duodenum, but if the opposite kidney was removed anorexia, nausea and vomiting developed within 48 hours, and the animals became progressively more emaciated and died after 7-12 days. They attributed these symptoms to absorption of some toxic substance from the urine. Goto (1918) showed that the blood non-protein nitrogen increased after unilateral ureteroduodenostomy in dogs and confirmed that removal of the opposite kidney resulted in death, but attributed these findings to impaired function of the kidney whose ureter was transplanted

Hinman and Belt (1922) investigated the matter further. They reported that bilateral ureteroduodenostomy, or unilateral ureteroduodenostomy combined with removal of the opposite kidney, resulted in symptoms resembling those produced by bilateral nephrectomy, and death within 12 days, but that the animal recovered if, within nine days of the operation, a ureter was disconnected from the duodenum and a cutaneous ureterostomy was established. They therefore concluded, like Baird *et al.*, that the symptoms were due to absorption of urinary constituents from the duodenum. Hinman and Belt reported further that after unilateral ureteroduodenostomy both kidneys hypertrophied at first but later the kidney draining to the duodenum became atrophic, and subsequently Hinman (1923, 1926a) used this observation as one of the foundations of his theory of renal counterbalance.*

Further indirect evidence in support of

*In brief, Hinman suggested that the atrophy is a form of disuse atrophy and can be explained by the fact that the kidneys normally work with varying intensity. When the normal one slacks it must make up for the deficiency later, whereas when the kidney connected to the duodenum slacks it is of no consequence. This hypothesis, and the evidence on which it was based, was later challenged by Bolliger and Walker-Taylor (1932).

to the result, but some urologists prefer always to make the segment isoperistaltic (Davis and Nealon, 1957)

Ileocystoplasty and Allied Procedures

In ileocystoplasty a pedicled isolated segment of ileum is used to increase the capacity of the bladder in patients with contracted bladder due to tuberculosis or other cause, *or following extensive partial cystectomy* According to Tasker (1953) this operation was first performed by von Mikulicz in 1898 and during the next thirty years occasional cases were reported (e g Scheele, 1923b) Sometimes both ends of the loop were closed and the centre was anastomosed to the bladder sometimes one end was closed and the other anastomosed to the bladder and sometimes the centre of the loop was anastomosed to the bladder and the ends were joined together to form a ring (Fig 195) Similar operations were also performed using segments of large bowel instead of ileum (*see* Pyrah and Raper, 1955)

Interest in these procedures was revived by Couvelaire (1950), who reviewed the whole problem and reported five cases in which he used a segment of ileum or caecum to increase the capacity of a tuberculous bladder, and many further cases of ileocystoplasty have since been reported by Cibert and his colleagues (Cibert, 1953, Cibert, Durand, Foret and Soler, 1954), Wells and his colleagues (Annis, Hunter and Wells, 1954), Pyrah (1954, 1956, 1957), Ferris (1955), and other surgeons

Uretero-entero-urethral Anastomosis

Lemoine (1913) performed cystectomy and anastomosed the ureters to the rectum in a patient with carcinoma of the bladder, and later, when ascending renal infection supervened, isolated the rectum and anastomosed it to the urethra He anastomosed the sigmoid colon to the anal margin, but

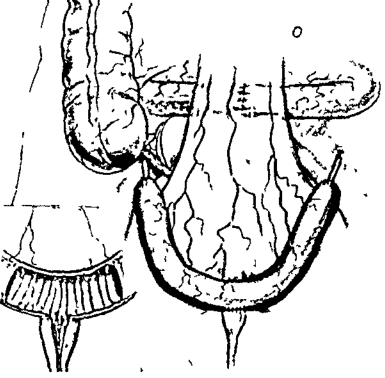


Fig 196 Pyrah's technique of uretero entero urethral anastomosis (Reproduced from the *Journal of Urology* by courtesy of the publishers and Professor L. N Pyrah)

this anastomosis broke down and the patient died eighteen days later.

More recently this type of procedure has been studied in dogs by Bisgard and his colleagues (Bisgard, 1943; Bisgard and Kerr, 1949), Rubin (1948, 1951) and Thompson (1950), who constructed a substitute bladder from an isolated pouch of sigmoid colon and anastomosed this to the urethra.

Couvelaire (1951), in a patient with carcinoma of the bladder, anastomosed the ureters to an isolated loop of ileum and, after performing total cystectomy, joined the distal end of the loop to the prostatic urethra and brought the proximal end out through the abdominal wall. Later he closed the fistula and the patient then passed all his urine *per urethram*. Micturition was produced by a combination of straining and manual pressure over the lower abdomen. A somewhat different technique was used more recently in two patients by Pyrah (1956, 1957), and in one patient by Raper (cited Pyrah, 1957), in which, following total cystectomy, the ureters were anastomosed to the ends of an ileal segment and the centre of the segment was anastomosed to the prostatic urethra (Fig. 196). A catheter was retained in the urethra for 10 days. Both of Pyrah's patients had cancer of the bladder. One died from pulmonary embolism four weeks after operation; the other made a good recovery and returned to work, but died two years later from recurrence of his tumour. Raper's patient was operated on because of recurrent Hunner's ulcer, and the operation appears to have been highly successful.

METABOLIC DISTURBANCES AFTER ANASTOMOSIS BETWEEN THE URINARY TRACT AND THE INTESTINE

Observations in Dogs

Ureteroduodenostomy

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the view that urinary constituents are absorbed from the duodenum after ureteroduodenostomy was obtained by Bollman and Mann (1935), who found that after removal of one kidney in dogs the remaining one increased in weight much more rapidly during the next few days if its ureter was anastomosed to the duodenum

The more recent work of Fortner and Kieffer (1948) has confirmed the earlier findings After unilateral ureteroduodenostomy the kidney concerned continues to function but its products are almost completely reabsorbed The blood non protein nitrogen increases, but the procedure is well tolerated provided that the opposite kidney is in good condition and able to compensate for the ineffectiveness of its fellow This tolerance is scarcely surprising in view of the observation of Geer and Dragstedt (1938) that continued drainage of the urine from one kidney to the blood stream by anastomosis of the corresponding ureter to a lumbar vein is also well tolerated

Ureterojejunostomy and Uretero-ileostomy

Bollman and Mann (1927) found that anastomosis of both ureters to the jejunum or ileum, or anastomosis of one ureter combined with removal of the opposite kidney, resulted in a rapid rise in blood urea without any accompanying rise in the blood creatinine creatine uric acid or amino acids and death within 14 20 days Unilateral ureterojejunostomy or uretero ileostomy caused no change in any of the non protein nitrogenous constituents of the blood

Ureterocolostomy and Cystocolostomy

Bollman and Mann (1927) found that anastomosis of both ureters to the colon proximal to the rectum caused a temporary rise in blood urea with a gradual return to normal in 6 10 weeks, while bilateral anastomosis to the rectum caused a rise in blood urea for only a few days It was shown later, however, by Jewett (1940) that fatal uraemia might occur after bilateral ureterocolostomy if the passage of urine *per anum* was long delayed

Boyce (1951) anastomosed the bladder to the colon at various levels and found that there was some absorption of Cl^- and of nitrogenous constituents of the urine The blood uric acid increased to about the upper limit of the normal range and the alkali reserve fell to about the lower limit of the normal range The degree of absorption was proportional to the surface area of bowel wall in contact with urine and the duration of contact The rate of absorption was greatest in the caecum and least in the rectum Diversion of all urine to the caecum resulted in death with a high blood non protein nitrogen and signs of uraemia Diversion of all urine to the sigmoid colon was not incompatible with prolonged survival, however, because the reserve of renal function was sufficient to compensate for the re absorption

In later experiments Boyce and Vest (1952) instilled a solution of NH_4Cl into an excluded portion of the dog's colon They found that both ions (NH_4^+ and Cl^-) were absorbed 'in proportion to their molecular ratio,' and in consequence suggested that *hyperchloraemic acidosis* following ureterosigmoidostomy in man (p 609) is due mainly to absorption of NH_4Cl, the ammonia being formed from decomposition of urine in the colon and subsequently converted in the liver to urea Boyce and Vest also pointed out that electrolyte disturbances due to reabsorption can be distinguished from those due to impaired renal function, because the sulphate ion (SO_4^{--}) is not absorbed from the bowel, and the phosphate ion (HPO_4^{--}) is absorbed to only a slight extent, whereas both are retained in renal failure

D'Agostino, Leadbetter and Schwartz (1953) investigated the capacity of the colon to absorb and excrete electrolytes by instilling 150 200 ml of isotonic NaCl into an excluded loop of colon and measuring the concentration of electrolytes in the plasma

and in the fluid in the loop at various intervals thereafter. They found that concentration gradients were established across the colonic mucosa, HCO_3^- being higher and Cl^- being correspondingly lower in the colon than in the plasma. As a rule also some Na^+ in the colon was replaced by K^+.

Rosenberg (1953) studied the absorption of radioactive isotopes of sodium (Na^{24}), iodine (I^{131}) and phosphorus (P^{32}) from the rectum in normal dogs and in dogs previously subjected to bilateral ureterosigmoidostomy, iodine being used because of the similarity in behaviour of the iodine and chlorine ions. In addition he compared the concentration of ammonia, chloride and urea, and also the pH, in blood from the sigmoid vein, the portal vein and peripheral veins, in dogs with bilateral ureterosigmoidostomy. It was found that Na^+ and Cl^- (or I^-) were absorbed equally well, and the absorptive power of the bowel was not altered by acidosis. Sigmoid venous blood was found to contain more ammonia, chloride and urea than peripheral venous blood, and also to be more acid. It was concluded that, since acidosis is compensated for by increased urinary excretion of ammonia and titratable acid, a vicious circle is set up, the sigmoid colon being presented with more and more acid salts, notably NH_4Cl.

Clinical Observations

It will be convenient to begin by considering the metabolic consequences of ureterocolostomy, because in man these have been studied for much longer than the changes which occur after anastomosis of the urinary tract to a segment of small bowel.

Ureterocolostomy

Boyd (1931) observed nitrogen retention and acidosis in a child aged 10 who had had bilateral ureterosigmoidostomy performed three years previously for ectopia vesicae. The child was poorly nourished and showed skeletal changes characteristic of rickets. The alkali reserve (CO_2 combining power of the plasma) ranged from 34 to 45 volumes per cent and the non-protein nitrogen was 67 mg./100 ml. He improved greatly with administration of milk, cod liver oil and sodium bicarbonate, and exposure to ultraviolet irradiation, but the alkali reserve remained persistently low, averaging 35 volumes per cent

Some years later Jewett (1944) reported acidosis and uraemia in one of 33 patients subjected to ureterosigmoidostomy. The condition developed three years after operation and was attributed to the fact that the patient evacuated urine from his bowel only thrice daily. He responded to enemas and administration of fluid* intravenously.

The first extensive investigation of the problem was made by Ferris and Odel (1950), who found evidence of hyperchloraemic acidosis ($Cl^- > 103$ mEq /litre; CO_2 combining power < 50 volumes per cent) in 80 per cent of 141 patients previously subjected to bilateral ureterosigmoidostomy. Subsequently large series of cases have been studied by Doroshow (1951), and Clarke and Leadbetter (1955), in the United States, and by Jacobs and Stirling (1952) in Britain Smaller series have been reported by Kekwick, Paulley, Riches and Semple (1951), Lapides (1951), Wilkinson (1952) and others.

The mechanism of hyperchloraemic acidosis after diversion of urine to the colon has been much disputed. The following three factors, either singly or in combination, have been held to be mainly responsible: absorption of chloride from the colon, impaired renal function, and excessive loss of potassium.

Ferris and Odel (1950) attributed the condition to absorption of Cl^- from the rectosigmoid, and treated it by restricting the intake of salt and administering sodium bicarbonate. In a further study Odel, Ferris

*Composition not specified

and Priestley (1951) confirmed and extended these observations. They found that administration of NaCl or NH_4Cl raised the serum Cl^- level but did not alter the level of Na^+, and therefore suggested that there was *selective* absorption of Cl^- from the rectosigmoid.

The dominant importance of chloride absorption has been stressed also by Doroshow (1951) who in a study of 115 patients with bilateral ureterosigmoidostomy found that there were 52 in whom it seemed possible to rule out gross impairment of renal function, among whom 27 showed transient azotaemia, acidosis and hyperchloraemia, and by Mitchell and Valk (1953), Rosenberg (1953), Bohne (1953) and others. Important experimental evidence in support of this view was obtained by Annis and Alexander (1952) who instilled fluid—consisting of either distilled water, physiological saline or the patient's urine—into the distal loop of a double barrelled colostomy in two patients both of whom had virtually normal bowel distal to the colostomy. The fluid was withdrawn after three hours and observations were made of its volume, pH, and content of sodium, potassium, ammonium chloride, bicarbonate, urea and uric acid. When urine was instilled there was no significant change in volume but the pH changed from a strongly acid to an alkaline reaction. The concentration (and total quantity) of sodium decreased, but there was a much greater fall in chloride, about half of which was absorbed and replaced by bicarbonate. There was no significant change in potassium content.

Kekwick, Paulley, Riches and Semple (1951) attributed the hyperchloraemic acidosis and other signs of disordered metabolism which they observed in five patients after ureterocolostomy to a lesion of the distal renal tubules. Lapides (1951) reached a similar conclusion. In a study of 22 patients subjected to ureterosigmoidostomy he observed a close relationship between the incidence of hyperchloraemic acidosis and evidence—clinical and pyelographic—of impaired renal function. In addition, as an experiment, in three individuals with normal renal function and in three others with poor renal function, he instilled their own urine into the rectum for periods up to 12 days. In those with normal renal function the blood urea and electrolyte levels remained normal. Of the three individuals with poor renal function, however, two developed hyperchloraemia, two developed acidosis, and all showed an increase in the blood urea. Within a few days of stopping the instillation the blood urea and electrolyte levels returned to normal. Lapides concluded therefore that healthy kidneys can compensate for the absorption of urea and electrolytes which occurs after ureterosigmoidostomy but kidneys whose function is impaired cannot.

Jacobs and Stirling (1952), in a study of 201 cases of ureterosigmoidostomy, found that 31.3 per cent showed hyperchloraemic acidosis associated with a raised blood urea whereas only 9.0 per cent showed hyperchloraemic acidosis with a normal blood urea. They formed the opinion that hyperchloraemic acidosis was due partly to absorption of chloride and partly to impaired renal function. A similar conclusion was reached by Pyrah and his colleagues (Parsons, Pyrah, Powell, Reed and Spiers, 1952; Pyrah, 1954).

Diefenbach, Fisk and Gilson (1951) observed marked hypokalaemia associated with acidosis in one patient in whom ureterosigmoidostomy had been performed 14 months previously. Subsequently Wilkinson (1952) found hypokalaemia (i.e. serum K $\leqslant$ 3.5 mEq./litre) in four out of seven patients with hyperchloraemic acidosis following ureterosigmoidostomy, and put forward the hypothesis that hyperchloraemic acidosis developing after this operation is the result of increased secretion from the colon, with consequent loss of potassium and water, and

compensatory retention of sodium and chloride. There is no doubt that hypokalaemia is not uncommon after ureterosigmoidostomy, and may be associated with hyperchloraemic acidosis (*see* e.g. Jacobs and Stirling, 1952, Skanse and Widén, 1955),* but there are also many cases of hyperchloraemic acidosis in which there is no associated hypokalaemia, and these are not accounted for by Wilkinson's hypothesis.

Increased blood urea after diversion of urine to the colon has usually been attributed to impaired renal function, absorption of urea, or a combination of these factors. There is evidence, however, that it may result from absorption of ammonia and subsequent conversion of this ammonia to urea in the liver (Mitchell and Valk, 1953; Weyrauch, 1956).

Anastomosis Between the Urinary Tract and a Segment of Ileum

Cases have been reported in which hyperchloraemic acidosis developed after uretero-ileostomy (Bricker, 1952; Wilson, 1953), but this is not common. In Bricker's series, for example, it was observed in only one patient out of 28 followed for six months or longer.

In an attempt to elucidate the reason for this Eiseman and Bricker (1952) instilled solutions containing sodium chloride and urea into isolated segments of ileum to which the ureters had been anastomosed. Every half hour they withdrew samples of fluid from the segment and also took blood samples for analysis. They concluded from their observations that both urea and chloride are absorbed from the ileum when present in the same concentration as in normal urine. More recently, however, Pyrah, Care, Reed and Parsons (1955) have shown that this is not the whole story. After instilling a solution containing radioactive isotopes of sodium (Na^{24}) and chlorine (Cl^{38}) into the bladder of a patient in whom bilateral ureterosigmoidostomy had been performed in conjunction with repair of a vesico-vaginal fistula, they found that Cl^- and Na^+ migrated at the same rate, and also that these ions migrated in both directions, i.e. from bladder to blood and *vice versa*, so that a dynamic equilibrium was set up. Subsequently they repeated their observations in the same patient after one ureter had been re-connected to the bladder via an isolated loop of ileum, and found no significant change in the migration of either ion. They concluded that as far as migration of Cl^- and Na^+ is concerned an ileal segment behaves very like the normal bladder and permits only a small net absorption of these ions in approximately equivalent amounts.

Further observations (Pyrah and Care, 1957) in which an isolated segment of ileum was perfused with various electrolyte solutions have shown that potassium ions behave somewhat differently. They too can migrate in both directions, but when the concentration of K^+ in the perfusing fluid is raised to approximately three times that in normal plasma—*i.e. about half that in normal urine*—there is a net absorption of potassium. If the renal function is normal, homeostasis of potassium can be achieved, but if it is not there is danger of hyperkalaemia (Pyrah, 1957).

*Jacobs and Stirling reported that hypokalaemia was found in 32 per cent of all patients examined six months or more after operation, and appeared to be due to polyuria or diarrhoea.

ANASTOMOSIS BETWEEN THE URINARY TRACT AND THE BOWEL IN PRESENT DAY SURGERY

DIVERSION OPERATIONS

Indications

The principal indications for diverting all the urine to the intestine are as follows

1 Congenital lesions
   (1) Ectopia vesicae
   (2) Epispadias or hypospadias associated with incontinence
   (3) Congenital neurogenic urinary incontinence
2 Traumatic lesions
   (1) Intractable vesico vaginal fistula
   (2) Post-operative sloughing of the bladder wall
   (3) Permanent incontinence due to injuries involving the vesical sphincter
3 Gross inflammatory contracture of the bladder not considered suitable for ileocystoplasty
   (1) Tuberculous
   (2) Non tuberculous
4 Primary carcinoma of the bladder
   (1) In conjunction with total cystectomy *
   (2) Without cystectomy as a palliative operation
5 Carcinoma of the other pelvic viscera involving the bladder
   (1) In conjunction with radical excision of the bladder and other pelvic viscera *
   (2) Diversion of urine alone as a palliative procedure

Choice of Procedure

The procedures which are most often used to divert all the urine to the intestine are as follows

1 Ureterosigmoidostomy without interruption of the continuity of the colon

2 Ureterosigmoidostomy with proximal colostomy

3 Anastomosis of the ureters to an isolated segment of ileum

In addition some surgeons still anastomose the ureters to an isolated ileo-caecal segment using the stump of terminal ileum as urethra This procedure has not gained general acceptance however because the patient has to be catheterized routinely (usually by himself) and this results in a high incidence of infection Moreover serious metabolic disturbances are likely to result if catheterization is not carried out frequently

It is difficult at the present time to formulate definite rules to guide the choice of procedure In 1956 Weyrauch expressed the then current opinion when he wrote We are now at the crossroads The individual surgeon may choose to sacrifice a perfect functional result in order to employ an exclusion method which may be fraught with fewer complications he may attempt to perfect an exclusion operation in which evacuation of urine is under some form of sphincter control or he may continue to strive for perfection in implantation to the intact intestinal tract treating re absorptive acidosis when it occurs by dietary measures or by a secondary exclusion procedure Since then however there has been something of a swing away from simple ureterocolostomy in favour of anastomosis of the ureters to the isolated rectosigmoid or to an isolated segment of ileum

According to Pyrah (1957) the late results of anastomosis to the isolated rectosigmoid have been appreciably better than those of the standard ureterocolic anastomosis in that the patients health has been more stable and the incidence of renal complications has been extremely low and the only disadvantage of the procedure is the colostomy Anastomosis to an ileal segment has proved

*The indications for total cystectomy are discussed in detail by Riches (1956)

very satisfactory in children with neurogenic urinary incontinence. It has also given some good clinical results in survivors when performed in conjunction with cystectomy, but both the mortality and the morbidity have been high, and the continuously draining urinary fistula is a decided disadvantage. Anastomosis to an isolated ileocaecal segment, as already mentioned, carries a considerable risk of ascending urinary infection and also of serious metabolic disturbances.

Technique

Ureterosigmoidostomy with or without Proximal Colostomy

The colon is prepared for operation by enemas, and by administration of sulphonamides or antibiotics or both to reduce the intestinal flora. Some surgeons rely on phthalylsulphathiazole or succinylsulphathiazole alone, but the combination of phthalylsulphathiazole and neomycin introduced by Bacon, Lowell, Spaulding, Rao and Trimpi (1954) acts more rapidly. Neomycin may also be used alone in a dosage of 1 g. every six hours by mouth. Given in this way neomycin appears to be completely non-toxic,* and eliminates pathogenic organisms from the stools in a day or two. Aureomycin or terramycin alone should not be used owing to the risk of staphylococcal enteritis due to resistant organisms.

The operative approach to the ureter may be transperitoneal or extraperitoneal according to circumstances, but in either case the anastomosis when completed is usually placed in an extraperitoneal position. The Coffey I (Fig. 187), Coffey II and Stiles (Fig 186) techniques, and minor modifications of them, are still currently used by many surgeons despite the rather high incidence of stricture formation and consequent hydronephrosis. The direct mucosa-to-mucosa anastomoses of Nesbit (Fig. 189) and Cordonnier (Fig. 190), which a few years ago seemed likely to displace indirect methods of anastomosis, in their original form have now been largely abandoned owing to the prohibitively high incidence of ascending infection. The combined technique of Leadbetter (Fig. 191), in which mucosa-to-mucosa suture is performed but in addition the lowest 2 cm. or so of the ureter is placed in a Coffey-type tunnel, is not open to this objection. Weyrauch's modification of Leadbetter's technique, in which the anastomosis is splinted by a catheter for seven days, gives even better results than the original procedure. The operation of Goodwin *et al.* (Fig. 192) achieves the same result as that of Leadbetter, and is technically easier to perform. It is probably the best method of anastomosis in use today

After operation, if ureteric catheters have not been inserted, urine should be drained from the rectum by an indwelling catheter for about a week. When the catheter is removed the patient should empty his bowel every two hours during the day and at least once during the night Fluid is usually given intravenously for a few days, the amount and nature of the fluid depending on the urinary output and on the electrolyte content of the serum and urine. Part of the sodium, and of the potassium also if required, should be given in the form of the lactate to avoid excessive intake of chloride. When the patient can take his full fluid and nutritional requirements by mouth his intake of chloride in the form of common salt is restricted and he is given a mixture containing sodium bicarbonate and potassium bicarbonate.

Renal infection, if it occurs, is usually best treated by administration of aureomycin, terramycin or chloromycetin. Pyrah (1954) advises that soluble sulphonamides should not be given since these inhibit carbonic anhydrase, an enzyme essential to the

*Neomycin given parenterally may be nephrotoxic and ototoxic.

[illegible] enhanced the renal [illegible]

Mild hyperchloraemic acidosis can often be corrected by increasing the oral intake of fluid, reducing the intake of chloride to 1 g daily or less, and administering sodium bicarbonate or sodium citrate. If the symptoms are more severe, fluid containing sodium lactate and, if there is hypokalaemia, potassium lactate in addition, is given intravenously. Frequent accurate determinations of the serum Cl, Na, and K levels and the CO_2 combining power are of course essential (see Pyrah, 1954, for illustrative cases).

Anastomosis of the Ureters to an Isolated Segment of Ileum

It is customary to carry out pre-operative treatment similar to that used before ureterosigmoidostomy, but it is doubtful whether much is gained by this.

The technique of the operation has been described in detail by Wells (1956). The ileal segment should be about 12 in. long and at least 8 in. of terminal ileum should be left. Reconstitution of the ileum, which is effected by end-to-end anastomosis above and in front of the isolated segment. Since the segment is open to the exterior as well as being isolated, there is no danger of reflux; the uretero-intestinal anastomoses can therefore safely be performed by direct mucosa-to-mucosa suture of the Nesbit type. The left ureter may be anastomosed to the inner end of the ileal segment and the right ureter to the antimesenteric border, or alternatively the inner end of the ileal segment may be closed and both ureters anastomosed to the antimesenteric border. The distal end of the segment is brought out through a stab wound to leave one inch protruding, after which the bowel is everted and the mucosa sutured to the skin. Urine is collected *ab initio* in a disposable ileostomy bag.

Post-operatively, intravenous infusion is necessary in the early stages, and is usually continued for 5–7 days.

REMODELLING OPERATIONS

Replacement or Reconstruction of the Ureter

When a ureter is divided, or occluded as a result of inflammatory or neoplastic disease, either some form of reconstruction or a diversion operation is necessary if the corresponding kidney is to be saved. Reconstruction by direct anastomosis of the ureter or by reimplantation into the bladder may be impossible, and in this event the most feasible method of reconstruction is to use an isolated ileal segment (Fig. 191). Replacement of part of a ureter by an ileal loop may also be required for ureteral fistula, and total replacement of both ureters has been used successfully for the treatment of megaureter.

When an ileal segment is used to replace the lower part of the ureter it may be placed either extraperitoneally or intraperitoneally. In either case the segment is best orientated so that it lies isoperistaltically.

When the whole of one ureter has to be replaced the ileal segment is placed intraperitoneally and is anastomosed end-to-side to the renal pelvis above and to the bladder below. When both ureters have to be replaced, one procedure is to anastomose the ends of an ileal segment to the renal pelves and the centre of the segment to the bladder, but some surgeons prefer to use more complicated procedures in which the drainage from both kidneys is isoperistaltic.

Ileocystoplasty

Ileocystoplasty is being used increasingly in preference to ureterocolostomy for the treatment of contracted bladder resulting from tuberculous or long-standing non-tuberculous *chronic cystitis*, and is also used occasionally to increase the capacity of the bladder after extensive partial cystectomy.

The various forms of operation have been illustrated in Figure 195. The length of ileal segment required depends on the capacity of the bladder, but usually ranges from 4-6 in. for a bladder with a capacity of 4-6 oz. to 12-15 in. for a tiny "thimble" bladder (Pyrah, 1957). When the bladder is very small Cibert (1953) and Pyrah (1957) advise excising the fibrosed detrusor muscle. If necessary one or both ureters may be divided and reimplanted in the ileal segment.

Pyrah (1957) uses two rows of continuous catgut suture for the anastomosis, the outer one seromuscular on the intestine and passing through the outer part of the muscle of the bladder, the inner one passing through the whole thickness of the wall of the intestine and the deeper muscle layer and the mucosa of the bladder. Peritoneum reflected from the bladder is sutured to the intestine in such a way as to make the anastomosis extraperitoneal. A drain is placed in the cave of Retzius and the bladder is drained by a urethral catheter for 10 days.

Uretero-ileo-urethral Anastomosis

As we have seen, it is possible to use an ileal segment as a bridge between the ureters and the urethra after total cystectomy. The procedure is attractive from the patient's point of view because if all goes well he continues to pass urine *per urethram*, though he may need to strain a little and also exert manual pressure over the lower part of the abdomen to achieve this. So far however only a few surgeons have had experience of this operation and it must be regarded as still *sub judice*.

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